Bed Bug Resistance to Selected Insecticides and Effect of Moisture on Efficacy of Selected Insecticide Dusts against the Common Bed Bug, *Cimex lectularius*

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ABSTRACT OF THESIS

Bed Bug Resistance to Selected Insecticides and Effect of Moisture on Efficacy of Selected Insecticide Dusts against the Common Bed Bug, *Cimex lectularius*

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Global resurgence of bed bugs, *Cimex lectularius* L. (Hemiptera: Cimicidae), due to increasing global travel, trade and resistance to different types of insecticides, is a very important concern today as it causes socioeconomic burden, mental stress and public health concerns. Insecticide resistance is considered as one of the key factors for the worldwide resurgence of bed bugs. Two studies were done to identify the resistance levels of bed bugs collected from different locations in New Jersey and Indiana and to understand the effect of moisture on the efficacy of insecticides against bed bugs. In the first study, I evaluated the bed bug resistance to commonly used insecticides Phantom SC (chlorfenapyr), Suspend SC (deltamethrin) and Transport GHP (acetamiprid + bifenthrin) using a vial assay and Transport GHP (acetamiprid + bifenthrin), Temprid SC (imidacloprid + β-cyfluthrin), and Tandem (λ-cyhalothrin + thiamethoxam) using a petri dish assay. Low to high levels of resistance were observed among the different strains of bed bugs. Resistance ratios of various strains based on LC_{50} were Transport GHP: 92-1,682, Temprid SC: 18-144, and Tandem: 179-1,226. In the second study, I evaluated the

effect of moisture on the efficacy of Cimexa (92.1% amorphous silica gel), Alpine (0.25% dinotefuran, 95% diatomaceous earth) and Tempo (1% cyfluthrin) against bed bugs. Moisture was created by two methods: 1. applying steam to insecticide dust treated panels and 2. aging the insecticide treated panels in chambers with various relative humidity (RH). Results show that exposure to moisture significantly reduced the efficacy of all tested insecticide dusts. The efficacy of the three insecticides was Cimexa > Alpine > Tempo. These results show that moisture needs be considered when applying insecticide dusts for controlling bed bug infestations.

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DEDICATION

To my father, Hari Charan Ranabhat and my mother, Shanta Ranabhat

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INTRODUCTION

The common bed bug, *Cimex lectularius* L. (Hemiptera: Cimicidae), is a blood-sucking urban pest that affects the quality life of people from different aspects as it causes socioeconomic burden, mental stress, and public health concern (Doggett et al., 2012; Eddy & Jones, 2011; Goddard & deshazo, 2009; Hwang et al., 2005). Due to its cryptic behavior and resistance to insecticides, it is one of the most difficult urban pest to control. Global resurgence of bed bugs has been observed in many countries in the last two decades, causing a major problem in human livelihood (Boase, 2001; Dang et al., 2017; Doggett et al., 2004; Potter, 2006; Romero et al., 2007; Wang et al., 2013). This resurgence is mainly due to insecticide resistance in bed bugs, frequent local and international travel, and lack of effective control materials (Doggett et al., 2004; Romero et al., 2007). Many researchers, pest management industries, federal agencies, and health departments are increasing their effort to address the global resurgence of bed bugs. Still, research on the current bed bug populations to better understand the behavior, biochemistry, genetics, and insecticide resistance mechanism to understand the fast-spreading of bed bug is scant. Therefore, more research is needed to develop improved tools and techniques to reduce the spread of bed bugs and manage bed bug infestations.

One of the major challenges in recent bed bug control strategies is the growing resistance of bed bugs to commercial insecticides. Bed bugs have shown the capability to rapidly evolve resistance mechanisms to escape insecticides toxicity. This includes behavioral and physiological changes to reduce the efficacy of insecticides. Monitoring insecticide resistance in bed bugs is very important in determining effective control

strategies. So, the purpose of my first study was to assess the resistance of different bed bug strains to commonly used insecticides using two methods: 1) vial assay and 2) petri dish assay. I evaluated bed bug resistance to commonly used pesticides: Phantom SC (chlorfenapyr), Suspend SC (deltamethrin) and Transport GHP (acetamiprid + bifenthrin) using a vial assay and Transport GHP (acetamiprid + bifenthrin), Temprid SC (imidacloprid + β-cyfluthrin), and Tandem (λ-cyhalothrin + thiamethoxam) using a petri dish assay. In this study, I hypothesized that different levels of resistance would be detected in different bed bug strains to different insecticides.

As dusts are easily picked up by bed bugs, dust formulations are considered more effective than spray formulations (Anderson & Cowles, 2012; Romero et al., 2009). However, the efficacy of dust can be affected by various factors like application methods and environmental conditions such as moisture where dusts are applied. It is very important to study and understand the role of moisture in the efficacy of insecticide dust against bed bugs for effective treatment. So far, there have been no reported studies on the effect of moisture on the efficacy of insecticide dust against bed bugs. In chapter three, I assessed the effect of moisture on the efficacy of three insecticide dust materials: Cimexa (92.1% amorphous silica), Alpine (0.25% dinotefuran + 95% diatomaceous earth), and Tempo 1% Dust (1% cyfluthrin), against bed bugs. Moisture treatments were done by applying steam using a steam machine or by aging the treated panels in chambers with 52, 75, and 100% RH. I hypothesized that the efficacy of selected insecticide dusts against the common bed bug will be reduced by moisture.

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CHAPTER 1: Literature Review

Background and History of *C. lectularius*

Bed bugs are very small flattened, brown-reddish hematophagous insects belonging to the order Hemiptera and family Cimicidae. The family Cimicidae includes 24 genera that consist of 110 species (Henry, 2009). Among them, 12 of the genera are associated with bats and 9 with birds. Only the genus *Cimex* has some species that are related to bats and others that occur on birds (Anderson & Cowles, 2012; Usinger, 1966). Three species, *Leptocimex boueti* (Brumpt), *Cimex hemipterus* F. (the tropical bed bug), and *Cimex lectularius* (the common bed bug) are known to feed on human blood. *Leptocimex boueti*, primarily feeds on bats but also feeds on human blood and found in West Africa. *Cimex hemipterus* feeds on humans, chickens and rarely bats and *Cimex lectularius* feeds on human, bats, chicken and other domesticated animals. The tropical bed bug is commonly found in the tropics and the common bed bugs occur all over the world (Usinger, 1966).

Bed bugs have been fossilized from archeological sites dating back more than 3500 years (Panagiotakopulu & Buckland, 1999). It is believed that bed bugs first parasitized bats and then later transferred to humans living in the same caves in the Mediterranean region (Panagiotakopulu & Buckland, 1999; Sailer, 1952). Later, when humans moved out from the cave, the bed bug also moved along with them. As expansion of civilization and commerce occurred, bed bugs spread throughout Europe, Asia and eventually America (Potter, 2011). Bed bugs were reported for the first time in 1583 in England and became common by the 17th and 18th centuries (Potter, 2011).

Bed bugs were reported in Italy by 77 C.E., China by 600 C.E., and Germany in the 11th century and France in the 13th century (Potter, 2011; Usinger & Povolny, 1966). Bed bugs were referenced from early writings from ancient Greeks, Egyptians, and Roman and also in religious writing as in New Testament and Talmud (Busvine, 1977; Kraft & Associates, 2007; Potter, 2011). The worldwide distribution of bed bugs can be traced to their naming also. Bed bugs were called *Cimex* (meaning 'bug') in ancient Rome and the species designation *lectularius* referred to a bed or couch. In ancient Greek, the term for bed bugs was *coris* which means "to bite". The early Spanish word for bed bug was *chinche* or *chinche de cama* which means "bug of the bed". Bed bugs were simply stated as "bugs" in England. Bed bugs have a variety of other descriptive names including "wall louse", "bed louse", "wallpaper flounder", "red coat", "crimson rambler" and "mahogany flat" originating from Europe and North America (Potter, 2011).

Pliny's Natural History mentioned that bed bugs had medical value to cure snake bites and ear infection. Some ancient people believed that bed bugs ingested with wine, beans, and other food could cure many diseases (Busvine, 1976). In North America and in Europe, bed bugs were used for medical purposes well into the 20th century (Potter, 2011). However, bed bugs became a serious pest which gave rise to various businesses to control bed bugs in England in the 17th century (Potter, 2011).

In the 18th and 19th centuries, for bed bug control, sprays mainly based on arsenic, mercury and pyrethrum were used, the first two being very toxic to humans (Potter, 2011; Usinger, 1966). Due to the lack of residual effect of these sprays, multiple insecticide treatments were needed for heavy infestations to eradicate adults and nymphs. More

effective ways to eradicate bed bug infestations were achieved with the fumigant sulfur and later in the beginning of the 20th century with hydro cyanide gas (Potter, 2011; Romero, 2011).

Besides residential areas, bed bugs were widespread in the non- residential areas such as schools, offices, theaters, hospitals, libraries, day care centers, retail stores, police stations, moving vans and funeral homes during the early 20th century (Potter, 2011). In the 19th century, to reduce the chance of people transporting bed bugs from old house to new, rigid disinfestation protocols were instituted in Europe. Families were taken to bed bug "cleansing stations," in England where their clothes and beds were passed through a steam disinfector. Citizens in Sweden were housed in tents while their buildings and personal belongings were fumigated (Potter, 2011). Some landlords in Germany required a written testimonial from exterminators stating that the apartments being vacated showed no signs of infestation (Hartnack, 1939; Potter, 2011). In a similar manner, today some property managers query about bed bugs during prescreening of new renters, though tenant rights are better defended than in the past. For example, in 2010, in New York city legislation was passed requiring leasers to provide the history of known bed bug infestation for the prior year to any renter before leasing the property (Cara, 2010).

Prior to World War II, bed bugs were extremely common pests in the United States. But the repetitive use of insecticides with residual effect, such as dichlorodiphenyltrichloroethane (DDT), organophosphate and carbamate radically reduced the bed bugs (Busvine, 1958). The demand for pest control services to control bed bug grew rapidly until the bed bugs were virtually eradicated through the widespread use of modern synthetic insecticides like DDT, and malathion in addition to improvement

in personal hygiene practices and sanitation in the middle of the 20th century (Kraft & Associates, 2007).

Bed bugs have again emerged as a global pest nearly after a fifty-year gap (Doggett et al., 2012; Pinto et al., 2007). There has been a worldwide resurgence of bed bugs, mainly in developed countries like Eastern Asia, Australia, Europe and North America (Boase, 2001; Doggett et al., 2004; Potter, 2005). Some pest management companies have reported that bed bug treatments increased more than 10 times (Cooper, 2006). Bed bugs have been reported in all 50 states in the U.S. within 5 years (Hedges & Moreland, 2004). In 2007, in an online survey among 509 pest control professionals surveyed, 91% reported that they had encountered bed bugs in the past two years; 37% reported they had encountered them five years ago and 21% had recalled seeing them more than 10 years ago (Potter et al., 2008). Bed bugs were found in schools, hospitals, theaters, retail stores, offices, libraries, moving the case during the early 20th century (Potter, 2010). At present, bed bugs are now considered as one of the most difficult urban pests facing today's pest management professional (Pinto et al. 2007; Potter 2011). Various factors considered for the sudden resurgence of bed bugs include increased national and international travel (movement of people and their belongings from the areas where bed bugs stayed common), development of insecticide resistance in bed bugs, use of second- hand furniture and other infested items (Boase, 2001; Doggett et al., 2004; Goddard & deshazo, 2009; Hwang et al., 2005; Maryanna et al., 2005; Romero et al., 2007). Also, other factors such as lack of awareness, lack of early detection, inadequate pest management, highly-priced pest control for the economically disadvantaged people help to spread of bed bugs throughout society (Pinto et al., 2007; Wang et al., 2011).

Biology and Lifecycle of *C. lectularius*

Cimicids or bed bugs that parasitize primarily humans, birds, and bats, belong to a highly specialized hematophagous taxon (Reinhardt & Siva-Jothy, 2007). Adult bed bugs can be easily observed and identified as dorso-ventrally flattened, elongated, light to reddish-brown 5 to 7 mm length insects (Figure 1.1). Bed bugs have three pairs of legs, short and board head with 4-segmented antennae, 3-segmented beak, two dark eyes and vestigial wings (Harlan, 2006b; Thomas et al., 2004). In males, the tip of the abdomen is pointed and in female, the abdomen's tip is rounded (Figure 1.1). Bed bugs have glands located in the ventral metathorax which produce a distinctive, musty odor containing various aldehydes (such as trans-hex-2-enal, trans-oct-enal) (Weatherston & Percy, 1978). Usually, bed bugs deposit undigested parts of earlier blood-meals in their hiding places that appears like tar or rusty residue (Figure 1.2).

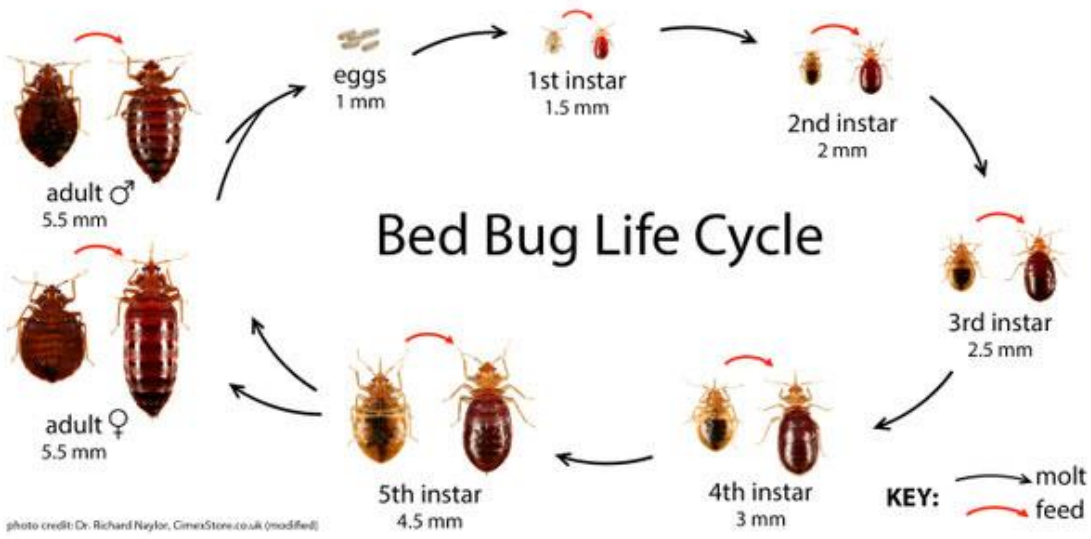

Figure 1.1. Lifecycle of *Cimex lectularius*

It takes 5 weeks to complete one life cycle from egg to egg at 28-32^{o}C and 75-80% RH (Harlan, 2006b). When the egg hatches, it undergoes through five nymphal instars and then molts to the adult stage. Nymphs require at least one blood meal during each instar before progressing to next (Usinger, 1966). C. *lectularius* are nocturnal but when they are hungry, they will feed during the daytime as well. In C. *lectularius,* feeding behavior coincides with periods of minimal host-activity (Reinhardt & Siva-Jothy, 2007). When they leave their refugia to feed, the host is mostly at rest or has minimal activity. It takes 10-20 minutes for an adult C. *lectularius* to fully engorge, once engorged it returns to its refugium (Usinger, 1966).

A B

Figure 1.2. Bed bug's eggs and its different stages. A. bed bugs cluttered on the edge of a bed sheet. B. bed bugs cluttered on the frame of a box.

Bed bugs mate by traumatic insemination (Harlan, 2006b; Stutt & Siva-Jothy, 2001; Usinger, 1966). For C. *lectularius,* feeding is an important prerequisite for mating. Adult males show their sexual interest more to recently fed females (Mellanby, 1939;

Reinhardt & Siva-Jothy, 2007; Siva-Jothy, 2006). Per feeding, females receive about five traumatic insemination where the male pierces the female's abdominal wall (Reinhardt & Siva-Jothy, 2007). During the copulation via traumatic insemination in bed bugs, the male pierces the abdominal wall of the female in a specialized region (ectospermalege) of the abdomen with their intromittent organ (paramere) (Carayon, 1966). Females also have a specialized para genial tract that functions in mating and through which sperm migrate to the eggs, while the genital tract is used during oviposition (Reinhardt & Siva-Jothy, 2007). Various factors like temperature (Usinger, 1966) (Johnson, 1941), food availability (Johnson, 1941; Kemper, 1930; OMORI, 1941; Polanco et al., 2011) and quality (Johnson, 1937) determines the development, fecundity, oviposition, and longevity of bed bugs.

Diseases Transmission

The possible role of bed bugs in human disease transmission has been subjected to many studies for centuries. Most of these studies were conducted on the common bed bug and the tropical bed bug (Burton, 1963). Bed bugs have been suspected to carry more than 40 human diseases but there is little evidence to show that such transmission has ever occurred (Burton, 1963; Goddard & deshazo, 2009). No evidence has been found that human pathogens can multiply within bed bugs, so that may explain why there are not any recorded cases of disease transmission by bed bugs bites (Lai et al., 2016).

Studies under the controlled laboratory condition showed that bed bugs are capable of transmitting *Trypanosoma cruzi*, the etiological agent of Chagas diseases and *Bartonella quintana*, the etiological agent of trench fever (Leulmi et al., 2015; Sentana-Lledo et al., 2015). But so far, there is no evidence to support that bed bugs transmit this

pathogen to humans in their natural habitat (Delaunay et al., 2011; Doggett et al., 2012; Goddard & deshazo, 2009; Koganemaru & Miller, 2013). Though different studies have reported that bed bugs are not capable of transmitting viral pathogens like HIV and Hepatitis B virus, arthropod-borne viral pathogens (such as Buggy Creek Virus, Fort Morgan Virus, and Kan Khoi virus) haven't been specifically investigated (Lai et al., 2016). Further studies are required to conclude whether bed bugs can indeed transmit human pathogens. One possibility is that bed bugs may contain 'neutralizing factors' that weakens pathogen virulence and that may reduce the capability of bed bugs to transmit infectious diseases (Lai et al., 2016).

Though bed bugs do not seem to cause human diseases, they reduce the quality of life by causing discomfort, anxiety, sleeplessness, and ostracism (Hwang et al., 2005). People that know they have bed bugs often suffer from anxiety, insomnia, nightmares as well as different types of mental health (Doggett et al., 2012; Goddard & deshazo, 2009; Goddard & de Shazo, 2012). Bed bug bites causes a wide range of reaction from no reaction, to swelling, intense itching, scarring of tissues and in rare systematic reaction like anaphylaxis (Doggett et al., 2012; Goddard & deshazo, 2009; Lai et al., 2016). In general, bed bug bites present as 2-5 pruritic, erythematous papules with central punctum (Goddard & deshazo, 2009). In some cases, itching caused by the bites of bed bugs can cause breaks in the skin that may result in secondary infection (Goddard & deshazo, 2009).

Bed Bug Control Methods

Pest management methods to control bed bugs can be generally divided into two broad groups: 1) non-chemical and 2) chemical methods. Non-chemical methods, as the

name suggests, does not include the use of chemicals for the treatment of bed bugs. This method relies on physical techniques to eliminate bed bugs like direct removal or killing of bed bugs using heat, steam, and vacuum (Figure 1.3). Using heat to control bed bugs has been applied since the 1900s where the infested room or whole building is heated to a temperature above the thermal death point of bedbugs (45°C) (Harlan, 2006a).Temperatures of 48.3 °C and 54.8°C were necessary to provide immediate mortality of adults and eggs, respectively (Kells & Goblirsch, 2011). Temperature below these lethal limits can also kill them but bugs and eggs need to be exposed for a sufficient amount of time. Effective heat treatment relies on temperature, time of treatment and relative humidity. Although an effective method to control bed bugs, heat treatment can damage home appliances, art-work, food, etc. Similarly, steam can be applied to kill live bed bugs and eggs in mattresses, upholstered furniture and other infested items that are not damaged by steam. Likewise, exposure to an extreme cold environment can kill bed bugs. Infested items placed in a freezer for 4 days at −17.8°C will kill bed bugs and their eggs (Olson et al., 2013).

Besides these temperature-dependent techniques, activities such as reducing clutter and hot laundering bed linens and infested clothing can also be fruitful in bed bug control. It was found that washing at 60°C and tumble drying on a hot cycle (> 40°C) for at least 30 minutes kill all life stages of bed bugs (Naylor & Boase, 2010).

Bed bug control by exclusion is another non-chemical method of bed bug control. Using mattress covers, placing pitfall style traps under furniture legs, placing double-sided tape to furniture legs can halt bed bug passage (Potter, 2006; Romero et al., 2017). Although some of these techniques can be rigorous and time-consuming, they can reduce

the need for chemicals and should be considered part of an effective bed bug management program.

Figure 1.3. Non-chemical methods to control bed bugs. A. Hot laundering and drying; B. Vacuum application; C. Steam treatment; D. Heat treatment.

Chemical control is widely used to control bed bugs because they are inexpensive and easy to apply. One of the major breakthroughs in controlling bed bugs occurred with the introduction of Dichloro-diphenyl trichloroethane (DDT), a chlorinated hydrocarbon in the 1940s (Potter, 2011). DDT was not only very effective against bed bugs at the time of treatment, but it also provided long residual insecticidal activity. Despite the high efficacy of DDT, within 10 years of its application, resistance to DDT began to appear in

bed bug populations. To tackle DDT resistance, other insecticides like 1% malathion in spray formulation, were effectively to control resistant bed bugs. Such modification in insecticides helped to clear bed bugs in public areas and households until the 1990s. In the early 1990s, a sudden resurgence in bed bugs was observed and now has become a common problem in households and public places in many parts of the world, including the United States (Koganemaru & Miller, 2013).

Chemical methods continue to one of the most commonly used methods for the control of bed bugs. However, their efficacy is decreased significantly by bed bug resistance to synthetic insecticides over the long haul. Inorganic insecticides such as silica-based desiccant dusts are not only environmentally friendly but also have been reported to slow down the development of insecticide resistance in bed bugs (Potter et al., 2014; Romero, 2011). While the advantage of using silica-based desiccant is immensely high, they are also limited by their allowable application sites.

For optimum results, integrating both methods of treatment based upon the bed bug-infested environment is necessary. IPM uses both chemical and non-chemical methods for effective and long-term solutions of bed bug infestations by careful designing and planning of action plans. IPM strategies include: 1) prevention, early inspection, and detection of site of infestation, 2) correct identification of infestation species, 3) education to the occupants of infested area about bed bug infestation and eradication strategy, 4) integrated application of chemical and non-chemical method of pest control based upon dynamics of infestation sites, and 5) follow up visit for efficacy evaluation and additional treatment if required (Cooper & Harlan, 2004; Koganemaru & Miller, 2013; Romero et al., 2017).

Insecticides against Bed Bugs and Their Mode of Action

The United States Environment Protection Agency (EPA) has registered more than 300 products for use against bed bugs which falls on basic seven chemical classes of insecticides: pyrethrins, pyrethroids, desiccants, biochemicals, pyrroles, neonicotinoids, and insect growth regulators (EPA, 2016). Pyrethrins and pyrethroids target the axon of nerve cells interfering with the sodium channel of the axon. Sodium channels are responsible for regulating nerve impulse. They act like an on/off switch for the transfer of nerve impulse from one nerve cell to another, thus maintaining normal neurological process. Pyrethrins and pyrethroids inhibit the sodium channel and delays the closing of channel resulting in uninterrupted firing of nerve impulses, thus causing instant death. Bed bugs exhibit tremors and shaking during this period (Suiter & Scharf, 2011).

Desiccants are insecticides with active ingredients like silica gels and diatomaceous earth with dehydrating characteristics. Silica gels are synthetic inorganic compounds composed of silicon dioxide. Whereas, diatomaceous earth is derived from microscopic fossilized algae diatoms and are skeletal remains composed of silicon dioxide. Both silica gel and diatomaceous earth act as a dehydrating agent by adsorbing thin wax layer of an insect exoskeleton. This results in increased exoskeleton permeability causing death by dehydration. Desiccants can be a good alternative to neurotoxins due to their desiccant mode of action and low toxicity to mammals. However, they are limited to dry (low humidity) environment and due to its light weight, are difficult to apply sometimes.

The biochemical class of insecticides are biologically derived materials with insecticidal properties. Cold-pressed neem oil is the biochemical insecticides registered

for use against bed bugs. Neem oils contain various compounds with insecticidal and medicinal properties. They can be a good alternative to synthetic chemicals. Because of the low toxicity to humans and animals, botanical insecticides including essential oils are considered to be less toxic than synthetic pesticides (Politi et al., 2017; Viciolle et al., 2012). As essential oil compounds pose a minimum risk, these compounds are exempt from full EPA registration (Federal Insecticides, Fungicides, and Rodenticides Act-FIFRA, 40 CFR 152.25) (USEPA). Some studies have shown that the plant- derived essential oils show contact and fumigant toxicity against field population of bed bugs (Feldlaufer & Ulrich, 2015; Politi et al., 2017; Zha et al., 2018). For the indoor use, more than a dozen essential oil-based products are commercially available but only two products EcoRaider bed bug killer (1% geraniol + 1% cedarwood oil + 2% sodium lauryl sulfate) and Bed Bug Patrol (0.003% clove oil + 1% peppermint oil + 1.3% sodium lauryl sulfate) caused > 90% mortality of bed bug nymphs when they were directly sprayed (Singh et al., 2014). In the field study, Eco Raider caused a similar reduction of bed bug as Temprid SC and a mixture of Temprid SC and Eco Raider (Wang et al., 2014). For pest control, the draw backs related to the use of essential oils are short residual life which requires frequent applications, high volatility that leads to odor problems and less field efficacy reported for different insect pest species (Isman, 2006; Regnault-Roger et al., 2012). Residual treatment of entomopathogenic fungus *Beauveria bassiana* has been shown to be pathogenic to bed bugs (Barbarin et al., 2012). These studies showed that the fungus was effective and also infected bed bugs could transfer the fungus to uninfected individuals. Fungal biopesticide Aprehend®, containing *Beauveria bassiana* was found effective against both insecticide-susceptible and insecticide resistant bed bugs (Barbarin

et al., 2017). Another entomopathogenic fungus *Metarhizium anisopliae* was shown to be a poor pathogen to control bed bugs, mostly at humidities that would likely to be encountered under field conditions (Ulrich et al., 2014).

Pyrroles are another class of insecticides that include chlorfenapyr as an active ingredient. Being a pro-insecticide, pyrroles differentiate itself from other classes of insecticides as their efficacy depends upon the enzymatic activity of the host. The mode of action of pyrrole insecticides is based upon destroying mitochondrial activity. Pyrroles target mitochondria and disrupts its activity of supplying energy to cells. Consequently, insects treated with pyrroles will slowly die due to lack of enough energy. Commercially, Chlorfenapyr is increasingly being used (Moore & Miller, 2006; Romero, 2011a; Wang et al., 2009). This chemical has shown varying performance against bed bugs. In a laboratory residual bioassay, Phantom (chlorfenapyr) was a relatively slow-acting (Moore & Miller, 2006), direct application of phantom on the walls of infested apartments resulted in 61% reduction in bed bugs population at 3 days (Moore & Miller, 2009). Based on laboratory studies, it has been reported that Phantom (chlorfenapyr) is a non-repellent insecticide with a long residual activity against bed bugs (Romero et al., 2010). On the other hand, some researchers reported poor performance of these products even with laboratory susceptible strains (Doggett et al., 2012). This product is relatively slow-acting and often combined with fast-acting materials (Potter et al., 2012; Romero et al., 2010)

Neonicotinoids are insecticides with active ingredients like imidacloprid, dinotefuran, thiamethoxam, clothianidin, and acetamiprid which are a synthetic form of the plant produced insecticide nicotine. Their mode of action is also on the nervous

system inhibiting acetylcholine (Ach) receptor. These receptors are responsible for controlling nerve stimulation. Neonicotinoids bind to ach receptor for a very long period (minutes) resulting in hyper-stimulation. As a result, bed bugs poisoned by these insecticides show tremors, hyperactivity, and eventual death.

Insect growth regulators are chemicals that includes juvenile hormone analogs and chitin synthesis inhibitors. They work by interfering with insect development by disrupting critical physiological functions associated with normal insect growth and development. They are effective against juvenile insects. Juvenile Hormone Analogs (JHAs), one type of the insect growth regulators, includes insecticides with active ingredients hydroprene, methoprene, pyriproxyfen, and fenoxycarb. JHAs prevent insects from maturing and blocks reproductive ability resulting in insect's death or sterilization. Another kind of insect growth regulator, chitin synthesis inhibitors (CSIs), includes insecticides with active ingredients diflubenzuron, hexaflumuron, noviflumuron, and lufenuron. CSIs disrupt the synthesis of chitin in insects which is a critical component of insect's exoskeleton. Lack of chitin can be fatal for bed bugs resulting in death (Suiter & Scharf, 2011).

Bed Bug Resistance Mechanisms against Insecticides

In the last 15-20 years, the global resurgence of bed bugs has been observed in many countries and caused major problems for human. This resurgence is largely due to the development of insecticide resistance in bed bugs, along with frequent global travel and poor pest management techniques. It is known that bed bugs have developed resistance to all major chemical insecticides used in the market. Understanding the

resistance mechanisms is imperative when selecting insecticides for bed bug management.

Insecticide resistance in bed bugs can be defined as an inherent property to survive the lethal concentration of insecticides acquired over a period of evolutionary changes. They are characterized by behavioral and physiological changes that can be inherited to younger generations (Lockwood et al., 1984). The first insecticide resistance in bed bugs was reported against DDT, one of the most widely used and effective insecticides in the 1940s. Afterwards, various classes of insecticides were used for controlling bed bugs. Despite these insecticides having different modes of action as discussed in the previous section, bed bugs developed resistance to all of them over time.

The mechanism of bed bug resistance is basically attributed to two major aspects- behavioral resistance and physiological resistance. Behavioral resistance is the change in behavior of insects to avoid insecticides. This can be stimulus-dependent or independent. Behavioral resistance has been reported well in cockroaches and other insects. German cockroach, *Blattella germanica* (L.) has been observed showing bait aversion to glucose in a stimulus-dependent behavioral change to avoid toxicant (Silverman & Bieman, 1993). Similarly, anopheline mosquito has been observed changing their regular behavior of biting inside to biting outside to avoid insecticides, as an example of stimulus-independent behavioral resistance (Gillies, 1956). In bed bugs, however, there has not been proper study and confirmation of behavioral resistance. The highly cryptic nature of bed bug which often hides in dark places has made this kind of study difficult for any confirmatory analysis. This may be the reason behind the contradictory observation of behavioral resistance observed in bed bugs, where one study reported bed bugs may

avoid insecticides treated surface and the other one reported no avoidance (Moore & Miller, 2006; Romero et al., 2009b). Although not rigorously confirmed by experiment in bed bugs, based upon studies in other insects, behavioral resistance may also be one of the mechanisms involved in insecticide resistance in bed bugs.

Physiological resistance in bed bugs, on the other hand, is widely studied and identified in bed bugs. Physiological resistance in bed bugs includes penetration resistance, metabolic resistance, and target site insensitivity. It has been reported that resistant bed bugs develop thick cuticle to prevent insecticides from entering inside the body (Dang et al., 2017; Koganemaru et al., 2013; Lilly et al., 2016). Penetration resistance is also often linked with metabolic resistance where bed bugs will get more time to detoxify the insecticides or remove the insecticides from the body before reaching the target site. Penetration resistance in bed bugs is attributed to the overexpression of cuticular protein genes that leads to overproduction of cuticle making a thick barrier layer. Two independent studies identified overexpression of at least sixty different cuticular protein genes (e.g. larval cuticle protein, pupal cuticle protein, chitin synthase, and chitin deacetylase) in insecticide-resistant common bed bug (*C. lectularius*) (Mamidala et al., 2012; Zhu et al., 2013).

Metabolic resistance is the major type of physiological resistance where bed bugs undergo a series of metabolic changes or modifications to detoxify insecticides. This is often accompanied by mutation in specific genes resulting in changes in proteins and enzymes. Three major enzymes linked with metabolic resistance in insecticides are P450s, esterases, and GSTs, accompanied by ABC transporters (Mamidala et al., 2011). P450s are a group of enzymes containing heme as a cofactor that plays an important role

in detoxification by oxidizing xenobiotics or foreign compounds to less toxic form for excretion. Elevated expression of P450s by elevated level of transcription of P450 genes have been observed in insecticide resistance bed bugs signifying the role of P450 in metabolic resistance of bed bugs (Mamidala et al., 2012; Zhu et al., 2013). Due to overexpressed P450s, insecticides are quickly bio-transformed to its less toxic form and excreted from bed bugs, resulting in low to none toxicity. The role of P450s in insecticide resistance was further confirmed by using its primary inhibitor piperonyl butoxide (PBO). Insecticide resistance in bed bugs was reduced significantly when PBO was used as a synergist (Romero et al., 2009b).

Another enzyme associated with metabolic resistance, esterases, is hydrolase that can convert esters into acid and water. Esterases are mainly involved in metabolic resistance of carbamates and organophosphates where it can quickly breakdown these chemicals into its non-toxic form. Insecticide resistant bed bugs have shown overproduction of non-specific esterases due to gene upregulation which can quickly sequester carbamates and organophosphates (Romero & Anderson, 2016; Zhu et al., 2013). Also, mutation in gene sequence responsible for esterase production has been observed in sheep blowfly resulting in the conversion of carboxylesterase to organophosphorus hydrolase, thereby conferring organophosphate resistance (Newcomb et al., 1997). This signifies that genetic mutation is also linked with insecticide resistance. However, this has not been reported in bed bugs. More studies in genetic level in bed bugs are required to confirm the possible role of mutation in resistance.

Glutathione S transferase (GST) is another major pathway for detoxification of xenobiotics. GST works by catalyzing conjugation and reduction reaction in xenobiotic

thereby increasing its solubility and facilitating the excretion. Higher GST expression was observed in the pesticide exposed population of bed bugs (Bai et al., 2011) suggesting a possible role of GST in the beg bug resistance mechanism. Based on the above-mentioned reports, it is evident that enzymes P450s, esterases, and GST are involved in bed bug resistance, basically by overexpression or genetic mutation. Besides these metabolic enzymes, a transporter- ATP-binding cassette (ABC) transporters, is also involved with bed bug resistance mechanism. ABC transporters are cellular checkpoints that regulate the flow of many substrates across the membrane. They regulate the import of nutrients to cells and exports of unwanted substances including toxins outside of the cell. They are responsible for the removal of toxin outside the cell and has been associated with resistance to major insecticides. A recent report suggested overexpression of ABC transported in insecticide-resistant bed bugs and confirmed the role of ABC transporters in bed bug resistance against pyrethroids (Mamidala et al., 2012; Zhu et al., 2013). Besides behavioral and physiological resistance, target site insensitivity is also one of the way bed bugs acquire resistance against insecticides. In this process, bed bugs make the insecticide target site insensitive thus disrupting the function of insecticides. This is often accompanied by mutation that makes the target site insensitive for insecticides but allows the normal function. Target site insensitivity mode of resistance has been widely observed in pyrethroids and other insecticides that act in the nervous system (Dang et al., 2017).

From these studies, it is clear that bed bugs resistance against insecticide involves complicated and multiple biochemical processes including genetic mutations, overexpression of detoxifying enzymes/transporters, and morphological changes that can

collectively lead to neutralize the effect of insecticides. Therefore, bed bug control methods need to consider all these complex insecticide resistance phenomena and warrants combined treatment methods to tackle highly resistant bed bugs.

Parameters Affecting Beg Bug Control

The environment of the infestations should be considered in bed bug management. Factors like humidity, temperature, light availability can significantly alter bed bug behavior and insecticides activity. For example, neurotoxin insecticides like pyrethroids and neonicotinoids, which can kill bed bugs instantly, can be unstable in moist conditions. Also, dust formulations like diatomaceous earth and silica gel can be less effective in moist conditions as moisture may interfere with the physical properties of the dusts. Substrate type of the infested rooms may affect the efficacy of insecticide sprays (Wang et al., 2016). Further study regarding the effect of environmental conditions on the efficacy of insecticides is required in order to design environment-specific treatment strategies for better bed bug control.

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CHAPTER 2: Assessing the Insecticide Resistance of Field Strains of the Common Bed Bug (*Cimex lectularius*)

Abstract

Development of insecticide resistance is considered as one of the key factors contributing to the worldwide resurgence of bed bugs, *Cimex lectularius* L. I evaluated the resistance of ten strains of bed bugs collected in New Jersey, to five commonly used pesticides using two methods. A vial assay was used to determine resistance levels to Phantom SC (chlorfenapyr), Suspend SC (deltamethrin) and Transport GHP (acetamiprid + bifenthrin) and a petri dish assay was used to determine resistance levels to Transport GHP (acetamiprid + bifenthrin), Temprid SC (imidacloprid + β-cyfluthrin), and Tandem (λ-cyhalothrin + thiamethoxam). In the vial assay, high resistance levels (< 30% mortality) to Suspend SC were found in Bayonne 2015, Canfield and Trenton strains; medium resistance (30-70% mortality) was found in the Elizabeth strain; a low resistance (>70% mortality) was found in the Hackensack and Dehart strains. In the vial assay, a high resistance level to Transport GHP was found in Bayonne 2015 and Trenton strains; medium resistance was found in Canfield, and Elizabeth strains; a low resistance level was found in the Dehart and Hackensack strains. Phantom was deemed ineffective against all bed bug strains. Based on LC_{50} from the Petri dish assay, the resistance ratios of strains Bayonne 2015, Canfield, Indy, Irvington 624-5G and Irvington to Transport GHP were 1628, 1554, 42, 1287 and 92 respectively. The resistance ratios of Bayonne 2015, Irvington 624-5G and Irvington to Temprid SC were 144, 86 and 18 respectively.

The resistance ratios of strains Bayonne 2015, Irvington 624-5G and Irvington to Tandem were 1226, 577 and 179 respectively. Bayonne 2015 was highly resistant to both Temprid SC and Tandem. This study revealed all tested strains had moderate to high resistance to the pyrethroid-neonicotinoid insecticide mixtures. The findings indicate that insecticide sprays may have limited effectiveness for the control of bed bugs when used alone.

KEY WORDS: bed bug, chemical, insecticide resistance, control

Introduction

The common bed bug, *Cimex lectularius* L. (Hemiptera: Cimicidae) is an obligate hematophagous insect that feeds on human. Bed bugs were part of everyday life until the wide use of DDT and other broad-spectrum pesticides in the 1940s and 1950s (Usinger, 1966). Bed bug infestations became rare in many industrialized countries due to the broad applications of these insecticides for decades (Potter, 2011a). Global resurgence of bed bugs has been reported over the past two decades (Boase, 2001; Doggett et al., 2004; Potter, 2006). Various factors are suggested for the sudden global resurgence of bed bugs including frequent exchange of second-hand items, an increase in local and international travel, poor pest management practice and insecticide resistance. Among these, insecticide resistance is one of the significant factors for the comeback of this pest (Romero et al., 2007b).

After a decade used of DDT, bed bugs developed resistant to these compounds (Busvine, 1958; Mallis & Miller, 1964). Over the last two decades, pyrethroid resistance was found widely spread in the bed bug populations across the United States (Romero et al., 2007a; Zhu et al., 2010). Multiple resistance mechanisms including reduced cuticular penetration, increased metabolic detoxification, and target site insensitivity (kdr type) have been detected in pyrethroid-resistant bed bugs (Adelman et al., 2011; Dang et al., 2017; Koganemaru et al., 2013; Yoon et al., 2008; Zhu et al., 2010).

The United States Environment Protection Agency has registered more than 300 products for use against bed bugs which belong to seven chemical classes of insecticides: Pyrethrins, Pyrethroids, Desiccants, Biochemicals, Pyrroles, Neonicotinoids, and Insect

growth regulators (EPA, 2016). Most field populations of bed bugs are resistant to pyrethroids (Davies et al., 2012) and juvenile hormone analogs (JHAs) are ineffective at the label rate for controlling bed bugs (Goodman et al., 2013). Chlorfenapyr (pyrrole class) is increasingly being used commercially (Moore & Miller, 2006; Romero, 2011; Wang et al., 2009). Phantom (chlorfenapyr), a proinsecticide acting on oxidative phosphorylation, killed pyrethroid resistant bed bugs (Romero et al., 2010), but its slow action prompted continued interest in other alternatives. Although resistance to chlorfenapyr has been not reported in bed bugs, the efficacy of this chemical varied (Dang et al., 2017). Phantom (chlorfenapyr) was a relatively slow-acting in a laboratory residual bioassay (Moore & Miller, 2006). Direct application of phantom on the walls of infested apartments resulted in a 61% reduction in bed bugs population at 3 days (Moore & Miller, 2009). Poor performance of this product has been reported even for laboratory susceptible strains (Doggett et al., 2012)

Neonicotinoids are widely used against chewing and sucking pests (Jeschke et al., 2010) including bed bugs (Dang et al., 2017; Gordon et al., 2014; Potter et al., 2012; Wang et al., 2015; Wang et al., 2013). In an effort to combat resistance, neonicotinoid insecticide combined with pyrethroids have been introduced in the US for bed bug control (Potter et al., 2012; Potter et al., 2011; Romero & Anderson, 2016). However, moderate to high level of resistance to neonicotinoids was shown in a recent study (Romero & Anderson, 2016). Furthermore, Gordon et al. (2014) showed a significant increase in the level of resistance to Temprid SC (imidacloprid + β-cyfluthrin) after selection of one generation Also, reduced susceptibility was reported in some field strains to chlorfenapyr and bifenthrin (Ashbrook et al., 2017). These studies suggest the need for

pesticide rotation or integration of both chemical and non-chemical approaches for sustained management of bed bugs populations**.**

Monitoring insecticide resistance status and resistance mechanisms in bed bugs is important to determine effective chemical control strategies. Once the resistance status of different field strains is known, resistance mechanism can be evaluated next. The purpose of this study was to assess the resistance of different field-collected bed bug strains to commonly used insecticides using two methods. Using a vial assay, I tested Phantom SC (21.45% chlorfenapyr), Suspend SC (4.75 % deltamethrin), and Transport GHP (22.73% acetamiprid, 27.27% bifenthrin. I used a Petri dish assay, to test Temprid SC (21% imidacloprid, 10.5% β-cyfluthrin), Tandem (11.6% thiamethoxam, 3.5% λ-cyhalothrin) and Transport GHP (22.73% acetamiprid, 27.27% bifenthrin). These insecticides are commonly used by pest management professionals in the U.S.

Materials and Methods

Bed Bugs

Nine field strains and a laboratory strain (Fort Dix) were used in this study (Table 2.1). Fort Dix is a laboratory strain that was last exposed to insecticides in 1973. Eight of the field strains were collected in apartment buildings in different cities in New Jersey during 2009-2018. One strain was collected in a high-rise apartment building in Indianapolis, Indiana. All these strains (Table 2.1) evaluated on this study have been maintained in the laboratory without any insecticide exposure since it was collected. Bed bugs were maintained in plastic containers of 5 cm diameter and 4.7 cm height (Consolidated Plastics, Stow, OH) with red folded card paper as harborages (University

Stationers Supply Co., Deerfield, IL). The containers were kept in an environmental chamber at 25 ± 1°C, 45 ± 10% RH, and a photoperiod of 12:12 (L:D) h. Females were not used in our any study in order to maintain the colony of the strain. The bed bugs were fed biweekly on defibrinated rabbit blood (Hemostat Laboratories, Dixon, CA) using a Hemotek membrane-feeding system (Discovery Workshops, Accrington, United Kingdom).

Experiment I: Vial assay evaluating bed bug resistance to Phantom SC, Suspend SC and Transport GHP.

Bed Bug Strains. Six field strains: Bayonne 2015, Trenton, Dehart, Hackensack, Canfield and Elizabeth were used in this experiment. These strains were never selected for resistance to any insecticide in the past. One day before exposure to insecticide, 10 males and 10 4^{th}-5^{th} instars nymphs were counted and transferred to clean plastic petri dishes (5.5 cm diameter and 1.5 cm height; Fisher Scientific, Pittston, PA) with a 1.5 cm diameter screened area on the lid. Females were not used in order to maintain the colony of the strain. Each petri dish was lined with filter paper (Fisher Brand filter paper size P8, 5.5 cm diameter) that was glued to the petri dish using a glue stick (Elmer's glue). In each strain, a total of around 240-400 bed bugs were tested. These strains used for the experiment were fed within 7 days of the experiment.

Insecticides. Phantom SC (chlorfenapyr), Suspend SC (deltamethrin) and Transport GHP (acetamiprid, bifenthrin) were tested in this experiment (Table 2.2). They were provided by manufacture. The concentration used was based on the label rate which

is 0.5%, 0.06% and 0.13% for Phantom SC, Suspend SC and Transport GHP, respectively.

Exposure method. A potter spray tower (Burkard Scientific Ltd., Herts, UK) was used to deliver the diluted insecticide to both sides of red construction paper. Both sides of the construction paper were sprayed at the rate of 3.9 mg/cm^2 or approximately 1 gallon/1000 ft^2. Water was applied to the construction paper in the control. The treated paper was then set aside in a drying rack to dry for 1-2 days and cut into strips (1.5 cm × 4 cm) on the day the experiment was conducted. The paper was then placed into a vial with an appropriate label indicating I, II, III and IV for Phantom SC, Suspend SC, Transport GHP and control respectively. Bed bugs were then transferred to the vials. Some strains (Canfield, Dehart, Elizabeth, Hackensack, and Trenton strains) had smaller population size, limiting the number of replicates per treatment to three, compared to the Bayonne 2015 strain, which was replicated four times per treatment. Vials were stored in an incubator in an environmental chamber at 25 ± 1°C and a photoperiod of 12:12 (L:D) h. Mortality was recorded hourly for 5 hours, then every 1-3 days until 7-14 days. A bed bug was considered dead if it did not show movement when it was prodded with forceps. Moribund bed bugs were those which could not crawl. Dead bed bugs were removed after each examination.

Experiment II: Petri dish assay determining resistance levels of bed bug strains to three commonly used pyrethroid-neonicotinoid mixture products.

Bed Bug Strains. The laboratory strain: Fort Dix and six field strains: Irvington 624-5 G, Bayonne 2015, Trenton, Canfield, Irvington, and Indy were used in this

experiment. These strains are maintained in the laboratory without any exposure to insecticide since it was collected. Similar to Experiment 1, one day before exposure to insecticide, 10 males and 10 4^{th}-5^{th} instars nymphs were counted and transferred to clean plastic petri dishes. The strains used for the experiment were fed within 7 days of the experiment.

Table 2.1. Bed bugs strains evaluated in the study.

Strain name	**Collection site**	**Year of Collection**
Fort Dix	Ft. Dix, NJ	1973
Indy	Indianapolis, IN	2008
Elizabeth	Elizabeth, NJ	2009
Dehart	Elizabeth, NJ	2011
Irvington	Irvington, NJ	2012
Irvington 624-5G	Irvington, NJ	2013
Hackensack	Hackensack, NJ	2013
Bayonne 2015	Bayonne, NJ	2015
Canfield	Canfield, NJ	2018
Trenton	Trenton, NJ	2018

Insecticides. Three insecticides were evaluated in this experiment: Transport GHP (acetamiprid, bifenthrin), Temprid SC (imidacloprid, β-cyfluthrin), and Tandem (λ-cyhalothrin + thiamethoxam) (Table 2.2).Temprid SC and Tandem were obtained from a Univar, USA andTransport GHP was obtained from manufacture A stock solution (ten times more concentrated than the label rate) was made for each insecticide (Transport GHP- 1.3%, Temprid SC- 0.75%, and Tandem- 1.0%). Preliminary tests were conducted to determine the range of concentration for each insecticide and different strains. Serial dilutions were then made to create varying concentrations. Six different concentrations of each insecticide that caused 20-100% mortality and one control group (water) were used for each insecticide. A Potter spray tower was used to deliver the insecticide evenly at the rate of 3.9 mg/cm^2 or approximately 1 gallon/1000 ft^2. A Petri dish with filter paper (Fisher Brand filter paper size P8, 5.5 cm diameter) was placed on the platform of the spray tower to receive the spray.

Table 2.2. Insecticides evaluated in the study.

Formulation	Trade Name	Active Ingredients	Manufacturer
Suspension concentrate	Phantom SC	21.45% chlorfenapyr	BASF Corporation, Florham Park, NJ, USA
Suspension concentrate	Suspend SC	4.75% deltamethrin	Bayer Crop Science LP, Research Triangle Park, NC, USA
Wettable powder	Transport GHP	22.73% acetamiprid, 27.27% bifenthrin	FMC Corporation, Philadelphia, PA, USA
Suspension concentrate	Temprid SC	21% imidacloprid, 10.5% β-cyfluthrin	Bayer Crop Science LP, Research Triangle Park, NC, USA
Emulsifiable concentration	Tandem	11.6% thiamethoxam, 3.5% λ-cyhalothrin	Syngenta Crop Protection, LLC, Greensboro, NC, USA

Exposure Method. Treated Petri dishes were allowed to dry completely before counted bed bug were transferred to the Petri dishes. Each concentration was replicated three times. There was a total of 21 Petri dishes per insecticide/strain combination (6

varying concentrations and 1 control). Mortality was checked at 24 h, 72 h, and 7 d by probing the bed bugs with featherweight forceps (BioQuip Products, Rancho Dominguez, CA). A bed bug was considered dead if it did not show movement, after being probed. Moribund bed bugs were those which could not crawl. Dead bed bugs were removed after each examination. Moribund and dead bed bugs were both considered dead when analyzing mortality.

Figure 2.1. Assays examining the residual efficacy of insecticide sprays against *C. lectularius*. A. Vial assay with treated paper, B. Petri dish assay with treated filter paper.

Data Analysis.

Corrected mortality was calculated using Abbott's formula (Abbott, 1925a). Analysis of variance (ANOVA) was used to analyze the percent corrected mortality data from each treatment in both experiments. The data were checked for normal distribution and transformed if they were not normally distributed. Tukey's HSD test was used to separate means of percent mortality. Probit analysis generated LC_{50} and LC_{90} for each

insecticide/population. The resistance ratio (RR = LC_{50} of the field strain divided by LC_{50} of the laboratory strain) was calculated for each strain of bed bug population. All statistical analyses were performed using SAS software (version 9.3) (SAS, 2011).

Results

Experiment I: Vial Assay Evaluating Bed Bug Resistance to Phantom SC, Suspend SC and Transport GHP.

Resistance level were determined based on the corrected mean mortality by day 7 (Table 2.3). The mortality in the control was ≤ 15% in all of the strains. Resistance level were classified as: High resistance - < 30% mortality, Medium resistance - 30%-70% mortality, and Low resistance - > 70% mortality. For Phantom SC, all of the strains including susceptible strain had very low mortality (< 20% at 7 d). Mortality caused by Phantom SC was not changed significantly at 14 d also. So, it was considered ineffective insecticide as a dry residue. The control mortality was high in some of the strain at 14 d, so we used 7 d observation to compare all strains.

Table 2.3. Susceptibility of selected field strains of bed bugs to three insecticides.

	Mean (± SE) corrected mortality at 7 d (%)			
Strain	**Transport GHP**	**Suspend SC**	**Phantom SC**	**ANOVA statistics**
Bayonne 2015	40 ± 3	18 ± 4	5 ± 3	F = 34.59; df = 2; P = 0.0001
Canfield	41 ± 4	2 ± 2	0	χ^2 = 6.79; df = 2; P = 0.034
Dehart	82 ± 7	92 ± 4	20 ± 8	F = 18.73; df = 2; P = 0.0026
Elizabeth	69 ± 3	69 ± 11	9 ± 7	χ^2 = 4.99, df = 2, P = 0.082
Hackensack	100 ± 0 %	79 ± 2	19 ± 9	F = 41.73, df = 2, P = 0.0003
Trenton	45 ± 13	2 ± 2	10 ± 10	F = 34.16, df = 2, P = 0.0005

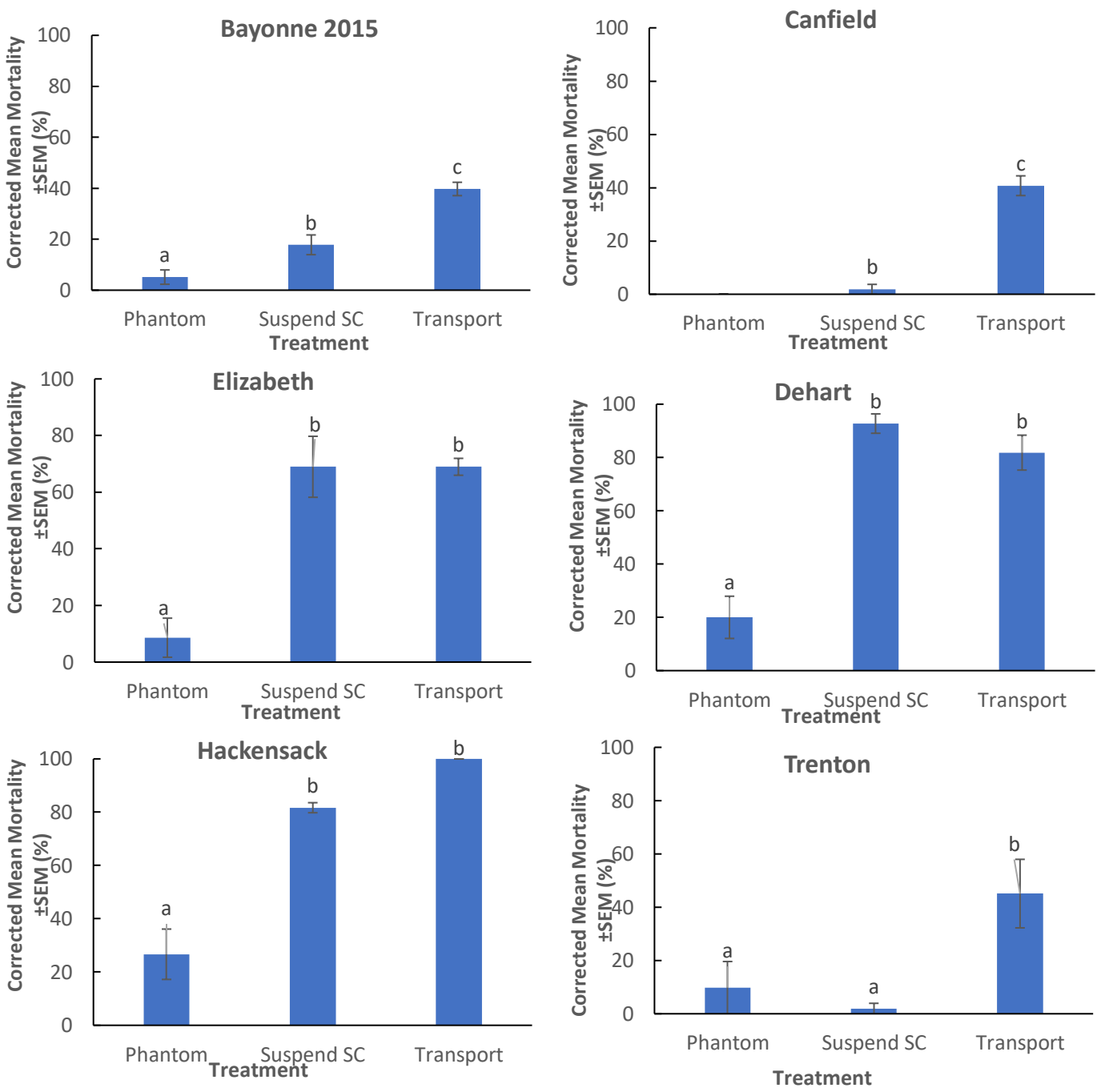

Figure 2.2. Corrected Mean Mortality (7 days) of different strains of bed bugs after continuous exposure to three insecticides using vial assay. Means with different letters are statistically different ($P < 0.05$, Tukey's HSD test)

Experiment II: Petri Dish Assay Determining Resistance Levels of Bed Bug Strains to Three Commonly Used Pyrethroid-Neonicotinoid Mixture Products.

In Transport GHP treatments, there was no mortality in the control at 72 hours for strains Irvington 624-5G, Bayonne 2015, and Canfield. The mean mortality in the control at 72 hours ranged from 7-15% for Fort Dix, Irvington and Indy strains. The Fort Dix strain was very susceptible to Transport GHP ($LC_{50} = 8 \times 10^{-4}$ mg/cm^2) (Table 2.4). All field strains exhibited significant resistance to Transport GHP compared to the laboratory strain (Fort Dix). The resistance ratio of Irvington, Irvington 624-5G, Bayonne 2015, Indy and Canfield strains relative to Fort Dix strain to Transport GHP were 92, 1287, 1628, 42 and 1554 respectively (Table 2.4).

In Temprid SC treatments, there was no control mortality at 72 hours for Irvington 624-5G and Bayonne 2015 strains. The mean mortality in the control at 72 hours for Irvington and Fort Dix strains was 2% and 5% respectively. Fort. Dix was highly susceptible to Temprid SC ($LC_{50} = 4.3 \times 10^{-3}$ mg/cm^2) (Table 2.4). The resistance ratio of Irvington, Irvington 624-5G and Bayonne 2015 strains relative to Fort Dix strain to Temprid SC were 18, 86, and 144 respectively (Table 2.4). Bayonne 2015 exhibited the highest degree of resistance to Temprid SC compared with other field strains.

In Tandem treatments, the mortality in the control was ≤ 3.33%. Significant differences were observed in susceptibility to Tandem between Fort Dix ($LC_{50} = 1.6 \times 10^{-3}$ mg/cm^2) and field strains. The resistance ratio of Irvington, Irvington 624-5G and Bayonne 2015 strains relative to Fort Dix strain were 179, 577 and 1226, respectively (Table 2.4).

Table 2.4. Concentration-mortality (72 hours) response of Cimex lectularius exposed to insecticide-treated filter paper.

Product	Active Ingredients	Strain	Slope±SE	LC_{50} mg/cm^2 (95% CI)	LC_{90} mg/cm^2 (95% CI)	RR_{50}	χ^2	df	p
Transport GHP	0.06% acetamiprid, 0.07% bifenthrin	Fort dix.	2.6 ± 0.3	0.0008 (0.0006-0.0010)	0.0024 (0.0019-.0036)	_	57.1	1	0.0001
		Bayonne 2015	1.7 ± 0.3	1.29 (0.79-1.82)	7.31 (4.59-17.27)	1628	34.9	1	0.0001
		Canfield	1.2 ± 0.2	1.23 (0.79-2.10)	14.84 (6.83-54.09)	1554	49.9	1	0.0001
		Irvington 624-5G	1.3 ± 0.2	1.02 (0.56- 1.51)	9.46 (5.34-28.94)	1287	32.2	1	0.0001
		Irvington	0.8 ± 0.1	0.07 (0.03-0.15)	3.79 (1.26-24.38)	92	49.5	1	0.0001
		Indy	0.7 ± 0.1	0.03 (0.02-0.06)	2.14 (0.76-12.00)	42	48.8	1	0.0001
Temprid SC	0.05% imidaclopri d, 0.025% β-cyfluthrin	Fort dix	1.8 ± 0.3	0.004 (0.003-.006)	0.02 (0.01-0.06)	_	31.6	1	0.0001
		Bayonne 2015	1.9 ± 0.2	0.62 (0.46-0.84)	2.96 (1.94-5.65)	144	73.2	1	0.0001
		Irvington 624-5G	2.5 ± 0.2	0.37 (0.31-0.44)	1.22 (0.96-1.67)	86	132.3	1	0.0001
		Irvington	0.9 ± 0.2	0.08 (0.03-0.18)	2.154 (0.70-19.01)	18	32.8	1	0.0001
Tandem	0.1% thiamethoxa	Fort dix	1.2 ± 0.1	0.0016 (0.001-.002)	0.02 (0.01-0.04)	_	135.2	1	0.0001

m, 0.03% λ-cyhalothrin	Bayonne 2015	2.2 ± 0.3	2.00 (1.62-2.55)	7.68 (5.21-14.92)	1226	53.4	1	0.0001
	Irvington 624-5G	2.0 ± 0.3	0.94 (0.70-1.26)	4.09 (2.76-7.67)	577	59.3	1	0.0001
	Irvington	1.5 ± 0.2	0.29 (0.20-0.41)	2.08 (1.31-4.07)	179	68.8	1	0.0001

Discussion

Very few effective classes of insecticide are available today for bed bug management. The predominant use of pyrethroid-based products has selected for resistance in many populations of bed bugs in different parts of the world (Gordon et al., 2014; Potter, 2010). The majority of the pest management professionals favor dual-action products containing both pyrethroid and neonicotinoids (Potter et al., 2013). Pyrethroids inhibit the sodium channel and delays the closing of channel resulting in uninterrupted firing of nerve impulse, thus causing shaking and instant death (Suiter & Scharf, 2011).

Neonicotinoids mimic the agonist action of acetylcholine at nicotinic acetylcholine receptors that results in overstimulation of the nervous system causing involuntary muscle contraction, cessation of feeding, paralysis, and ultimately leads to death (Bloomquist, 2009; Suiter & Scharf, 2011). Because pyrethroid and neonicotinoids insecticides have a different mode of action, a combination of these products produces greater control against pyrethroid-resistant populations (Potter et al., 2012). This study revealed that some bed bug strains have developed very high levels of resistance to pyrethroid-neonicotinoid mixture products. It provides evidence that it is necessary to

change the bed bug management practices to slow down resistance development and develop more alternative materials and methods to control bed bugs.

Various studies reported multiple resistance mechanisms, including penetration resistance by thickening or remodeling of cuticle, metabolic resistance by increased activities of detoxification enzymes and knockdown resistance by kdr mutations in bed bugs (Dang et al., 2017). Also, there are differently expressed genes including metabolic genes (P450s, esterases, ABC transporters and cuticular protein genes that are associated with pyrethroid resistance in bed bugs (Zhu et al., 2013). Romero and Anderson (2016) found that metabolic resistance might be involved in resistance to neonicotinoid as a result of the evaluation of activities of metabolic detoxification enzymes (P450s, GSTs, esterases) (Romero & Anderson, 2016). Further investigations of those strains which developed high levels of resistance to insecticide mixtures will be valuable.

In both experiments, different levels of resistance were found in different strains. Results indicate it is necessary to select insecticides and management practices based on the resistance profile of bed bug populations. Although Irvington and Irvington 624-5G were collected from the same building, Irvington was more susceptible to different chemicals compared to Irvington 624-5G. The Irvington strain was from multiple apartments, whereas the latter was from a single apartment. This demonstrates that within a building, there can be significant differences in levels of resistance among the bed bug infestations. Therefore, even within the same building, different units may need different treatment methods to successfully eradicate bed bugs.

Strain Irvington 624-5G was collected in 2013, six years prior to the study and they were reared in the laboratory without any insecticide exposure or selection. Still high resistance was observed in this strain to the insecticides evaluated in the study. This indicates that individual strain can maintain high resistance even in the lack of selection of resistance for many years. However, Gordon et al., (2014) suggested that in the absence of selection pressure, populations of bed bugs should revert towards increasing susceptibility. On the other hand, the susceptibility that has been observed in the laboratory maintained strains of bed bug suggest that resistance conferring genes may not be fixed within all population of bed bugs (Gordon et al., 2014).

In my study, dry residue of Phantom SC was deemed ineffective. Substrate type is one of the major factors that impact the residual efficacy of insecticide (Chadwick, 1985; Rust, 1995; Wang et al., 2016). Formulation is equally important. My experiment evaluated dry residue of Phantom SC on a piece of construction paper as a substrate. A previous study evaluating Phantom aerosol applied on various substrates found Phantom aerosol was effective on the substrate of fabric and unpainted wood, but it was not effective on a vinyl substrate. Further, studies are recommended to evaluate the efficacy of this products by using different substrate.

I observed Irvington strain was much more resistant to Tandem than Transport GHP and Temprid SC. Whereas, Irvington 624-5G strain was much more resistant to Transport GHP than Tandem and Temprid SC. The Bayonne 2015 strain was highly resistant to Transport GHP and Tandem and was much less resistant to Temprid SC. These variations in resistance could be due to different exposure history from the place where they collected, types of resistance mechanisms involved in each strain, and

composition of the insecticides. Though these three insecticides contain both a pyrethroid and neonicotinoid, they each contain different active ingredients in different amounts. Different specific active ingredients combination at different ratio may act differently.

Some limitations were observed in the vial assay study. Bed bugs often dropped to the bottom of the vials and therefore, exposure was not always continuous before the bed bugs died. Bed bugs falling off from treated papers is mostly due to knockdown effect. I observed healthy bed bugs were always able to cling to the treated paper or reattach to the treated paper after falling off the paper. During the initial a few hours of experiment, vials were rotated to allow dropped bed bugs to re-attach to the treated paper soon after falling off. Bed bugs that dropped from treated paper may also be due to repellency of the insecticide. Therefore, the vial assay method may not be suitable for highly repellent insecticides. In the Petri dish assay, sometimes bed bugs were clustered, and some bed bugs stay on top of others and could avoid continuous exposure to treated filter paper. Bed bugs were separated by gently blowing air with mouth to allow bed bugs to become more scattered. Because dead bed bugs were taken away after each examination and I used mortality after a few days of exposure, the variances due to discontinuous exposure was minimal.

From this study, most of the field bed bug populations evaluated in the study were found to be resistant to pyrethroid and pyrethroid-neonicotinoid mixture products, to varying degrees. Relying solely upon chemical control to control bed bug infestations will be difficult. Treating bed bugs with multiple insecticides with different modes of action,in combination with non-chemical methods should be the key approach to manage bed bug infestations and slow down insecticide resistance development. Further study of

the resistance prevalence and resistance mechanisms should be conducted to help understand the performance of the current insecticide products and design more effective methods and policies to help slow down the resistance development of bed bug populations and achieve greater control of bed bugs infestations.

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CHAPTER 3: Effect of Moisture on Efficacy of Selected Insecticide Dusts Against the Common Bed Bug, *Cimex lectularius*

Abstract

Dust formulations are considered as more effective for controlling bed bugs (*Cimex lectularius* L) (Hemiptera: Cimicidae) than residual sprays, as dusts are more easily picked up by bed bugs. However, there are various factors that can affect the efficacy of insecticide dusts. In this study, we evaluated the effect of moisture on the efficacy of selected insecticide dusts against *Cimex lectularius* L. Moisture was created using two methods: applying steam to insecticide dust treated panels and aging insecticide treated panels in chambers with various relative humidity (RH). In the steam treatment, three insecticides Cimexa (92.1% amorphous silica gel), Alpine (0.25% dinotefuran, 95% diatomaceous earth) and Tempo (1% cyfluthrin) were evaluated. Exposure to steam significantly reduced the efficacy of all three insecticide dusts. The efficacy of the three insecticides was Cimexa > Alpine > Tempo. In the different RH treatment, Cimexa treated panels that were aged under different (52%, 75%, and 100%) RH for one and two months caused significantly lower mortality to *C. lectularius* than dry Cimexa. Results show that exposure to moisture significantly reduced the efficacy of all tested insecticide dusts. Moisture needs to be considered when applying insecticide dusts for controlling bed bug infestations.

Key words: bed bug, insecticide dust, moisture, efficacy

Introduction

The common bed bug, *Cimex lectularius* L. (Hemiptera: Cimicidae) is one of the most difficult urban pests to control. Although there are many control materials and methods developed for the pest management industry, insecticide treatment remains the most popular for the management of bed bugs (Potter et al., 2013b). Nonchemical methods such as proper inspection, decluttering, vacuuming, encasement of mattresses and box springs, steam treatment, and structural heat treatment are all effective methods and should be considered in the development of a bed bug management program (Doggett, 2007; Kells, 2006; Wang et al., 2009). However, nonchemical methods cannot provide residual protection and may be expensive (Wang et al., 2009). Chemical treatment is often more economical and convenient compared to other bed bug control methods (Wang et al., 2015). For these reasons, insecticides are almost always used as part of the management of the program (Doggett, 2007).

Both liquid sprays and insecticide dusts are used for bed bug control. Dust formulations tend to offer better residual protection than pesticide sprays because they are more readily picked up by bed bugs (Anderson & Cowles, 2012; Potter et al., 2008; Romero et al., 2009). There are few laboratory studies available documenting the effectiveness of dust insecticides. Three insecticide dusts; Delta Dust (0.05% deltamethrin), Drione (1% pyrethrin, 10% piperonyl butoxide, and 40% amorphous silica gel), and Tempo 1% Dust (1% cyfluthrin) were evaluated by confining bed bugs to dust-treated surfaces continuously (Anderson & Cowles, 2012; Romero et al., 2009). Confinement of bed bugs on a treated surface provides a good indication of product efficacy but is not representative of field conditions, where it is unlikely that bed bugs

will continuously remain on dust treated surfaces. A recent study by Singh et al. (2016) reported high efficacy of silica gel dust among eight different commonly used insecticides using different exposure methods: brief exposure, forced exposure and choice exposure. Only silica gel dust caused 100% mortality from all exposure methods (Singh et al., 2016).

Most of the available insecticidal dusts contain pyrethroid or pyrethrin as active ingredients. However, a widespread resistance to pyrethroid insecticides has been reported in bed bugs (Romero et al., 2007; Zhu et al., 2013; Zhu et al., 2010). Further, a recent study showed that insecticide mixtures containing a pyrethroid and a neonicotinoid can quickly lose efficacy after repeated applications (Gordon et al., 2014). To slow down insecticide resistance development and control pyrethroid-resistant bed bugs, inorganic dusts (silica gel or diatomaceous earth dust [DE]) are suggested to manage bed bugs (Romero, 2011). In a field study, diatomaceous earth dust based IPM caused more bed bug reduction than chlorfenapyr spray based IPM (Wang et al., 2009). Until now, only two preliminary field studies have been reported where DE (Potter et al., 2013) or silica gel (Potter et al., 2014) were used alone to control bed bugs. Silica gel dust was found highly effective and was more effective than DE for controlling *C. lectularius*.

Various factors may affect the efficacy of insecticidal dusts used against bed bugs such as application methods and the conditions of the environment where dust is applied. Among these, moisture can be one of the major factors. There are very few studies evaluating the effect of moisture on the efficacy of insecticides. Increases in the LC_{50} by 6 to 8 fold were found when red flour beetles were exposed for 1 week on silica treated wheat and held at moisture contents of 11 compared with 16% (equivalent to 40 to 85%

RH) (Le Patourel, 1986). Toxicity was decreased when confused flour beetle were exposed on wheat treated with silica dust and held at increasing relative humidity (Aldryhim, 1990). Variation in toxicity of silica against German cockroaches was reported in partially treated choice boxes with relative humidity and was shown to be effectively eliminated at 95% RH or when free water was provided (Le Patourel & Zhou, 1990). To the best of my knowledge, there have been no reported studies on the effect of moisture on the efficacy of insecticide dust against the bed bugs. Insecticidal dusts can be subject to moisture both indirectly, via the indoor relative humidity or directly, via common treatment methods, such as liquid spray applications or the application of steam. For these reasons, it is important to study and understand the role of moisture in the efficacy of insecticide dust against bed bugs for effective treatment In this study, I evaluated the efficacy of selected insecticide dusts under different moisture treatments by applying steam and by aging the treated panels in chambers with 52-100% RH. The results will help make appropriate decisions when selecting insecticidal dusts for controlling bed bugs.

Materials and Methods

Bed Bugs

Bed bugs (Irvington strain) were collected from an infested apartment in Irvington, New Jersey in 2012, and was moderately resistant to pyrethroid insecticides in our preliminary laboratory assay They were maintained in plastic containers (5 cm diameter and 4.7 cm height; Consolidated Plastics, Stow, OH) with red folded paper as harborages (4 cm long and 3 cm wide; University Stationers Supply Co., Deerfield, IL) which were stored in an environmental chamber at 25 ± 1°C, 45 ± 10% RH, and a

photoperiod of 12:12 (L:D) h. The bed bugs were fed biweekly on defibrinated rabbit blood (Hemostat Laboratories, Dixon, CA) using a Hemotek membrane-feeding system (Discovery Workshops, Accrington, United Kingdom).

Dust Insecticides

Three insecticide dusts representing different classes of insecticides were included in this study. They were: inorganic dust- Cimexa (92.1% amorphous silica, 6.1 g/m^2, Rockwell labs ltd, North Kansas City, MO), neonicotinoid and inorganic mixture- Alpine (0.25% dinotefuran + 95% diatomaceous earth, 9.8 g/m^2, BASF Corporation, St. Louis), and pyrethroid- Tempo 1% (1% cyfluthrin, 4.9 g/m^2, Bayer Environmental Science, RTP, NC) dust. These insecticides are among the most commonly used products by professionals for bed bug treatments in the U.S (Potter et al., 2014b). They were obtained from the manufacturers.

Experiment I: Effect of Steam on Efficacy of Insecticide Dusts

The non-smooth side of vinyl tiles (15 by 15 cm) (Armstrong Civic, Armstrong World Industries Canada Ltd., Montreal, QC, Canada) were used as the substrate material. A 2.54 cm wide border was marked on each side of the tile (total area: 129.032 cm^2). An insecticide dust was applied to each of the four 2.54 cm wide borders of the tile using a fine brush. A total of 0.079 g of Cimexa, 0.116 g of Alpine and 0.063 g of Tempo was applied based on the label rate of each product.

A bucket (21.2 cm × 26.1 cm × 36.9 cm) was used to expose insecticide treated panels to steam. A 4.4 cm diameter hole (slightly bigger than the steam machine hose) was created on the wall of the bucket using a drill. Steam was applied using a steam

machine (The Steamax, Amerivap Systems, Dawsonville, GA) into the bucket through the hole on the wall. The bucket was covered with a lid that was slanted to allow steam to escape and prevent dripping from the lid to the panel placed at bottom of the bucket. The insecticide-treated tiles were placed on a plastic dish. The tiles were exposed to constant steam for 1 minute and 30 seconds at low-pressure setting and then allowed to rest in the chamber for 10 seconds before being removed and stored. A total 10 mg/cm^2 moisture was deposited per panel. Tiles were then allowed to dry for 24 hours in the laboratory (24 ± 1°C and 45 ± 10% RH) before they were exposed to bed bugs. The control panels were treated with insecticide dust but were not exposed to steam. In addition, a set of tiles not treated with any dust were used as negative control. Each insecticide dust treatment had five replications.

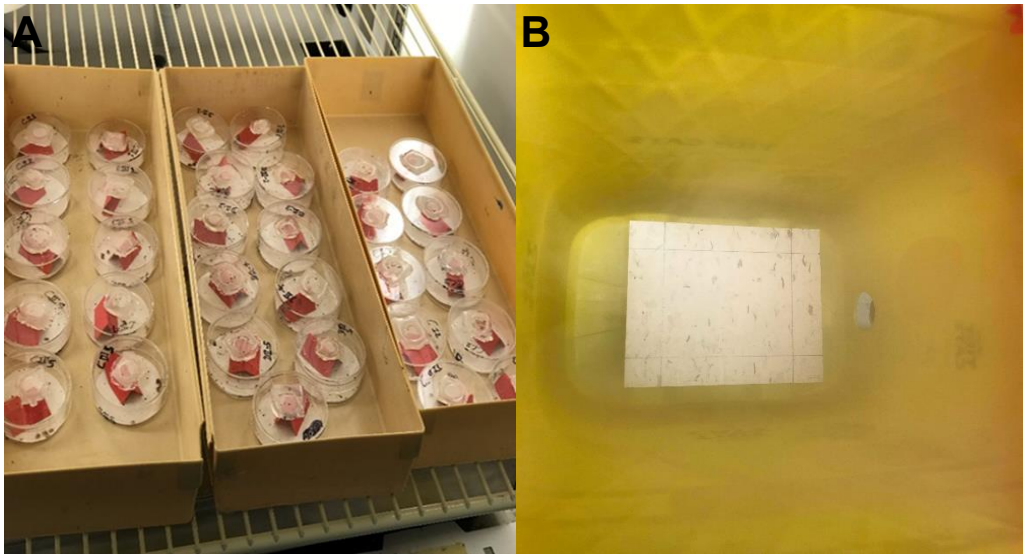

Figure 3.1. Experimental setup in the steam treatment. (A) Dishes with counted bed bugs, (B) A tile in a humidity chamber.

Two days before exposure to insecticide dust treated panels, 15 males and 10 3rd-5th instars nymphs were counted and transferred to clean plastic petri dishes (5.5 cm diameter and 1.5 cm height; Fisher Scientific, Pittston, PA). Females were not used in the study in order to maintain the colony. Each petri dish was lined with filter paper (Fisher Brand filter paper size P8, 5.5 cm diameter) that was glued to the petri dish using a glue stick (Elmer's glue) with red folded construction paper (3 cm long and 1.5 cm wide; University stationers supply Co., Deerfield, IL) to serve as a harborage on top of the filter paper. Bed bugs were fed eight days prior to the experiment.

Each tile was placed on a cardboard paper inside a clear plastic box. Twenty-five bugs were confined in the center of the tile with a plastic ring (9 cm diameter and 1.8 cm height) for 5 min to acclimate. The plastic ring was then removed, and bed bugs were allowed to cross the dust band (Figure 3.1). Once a bed bug crossed the treated band, it was removed using featherweight forceps (BioQuip Products, Rancho Dominguez, CA). The first 20 bed bugs that crossed the treated band were collected and placed in a petri dish. The time taken to cross the treated band was recorded for 20 bed bugs for each replication. It took 0.45 to 8.5 minutes to cross the dust band (from the first bed bug touching the inner border of the band to the 20th bed bug crossing the outer border of the band) The dishes were stored in an incubator at 25 ± 1°C, 45 ± 10% RH and 12:12 (L:D) h photoperiod. All handling of bed bugs and treatments were conducted inside the bin with walls covered with a thin layer of talcum powder to prevent any bugs from escaping. All experiments were conducted in a nonventilated room at 24 ± 1°C and 45±10% RH.

Bed bug mortality was recorded daily at 1-7, and on 10, 14 and 21 days after exposure. A bed bug was considered dead if it did not show movement when it was

prodded with featherweight forceps. Moribund bed bugs were those which could not crawl. Dead bed bugs were removed after each examination.

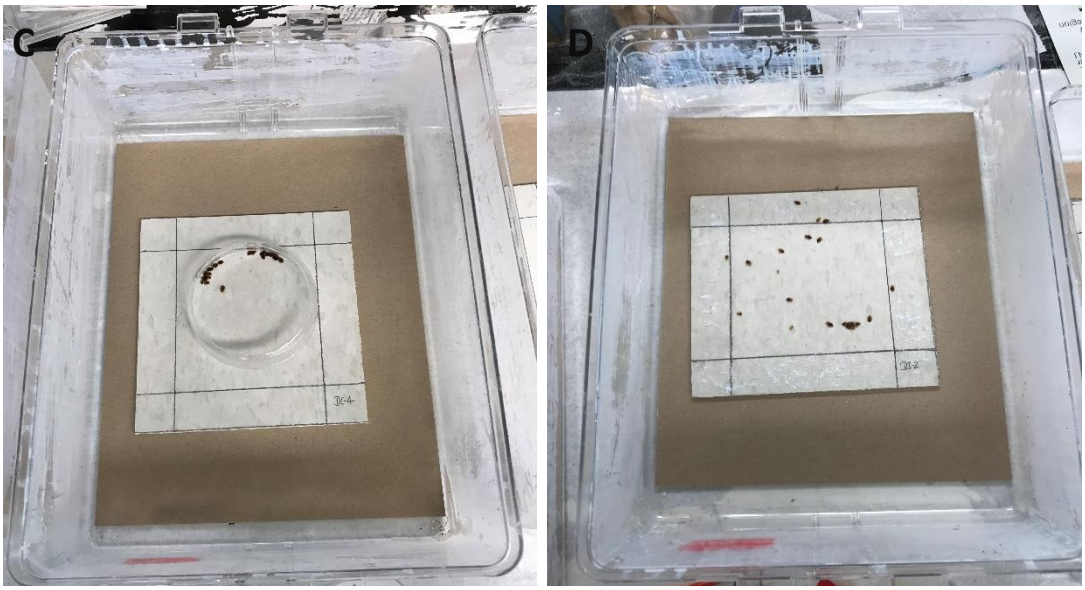

Figure 3.2. Experimental setup in the steam treatment experiment. (C) Bed bugs were confined in the center of a 15 by 15 cm vinyl panel with a plastic ring, (D) Bed bugs on a panel with a plastic ring removed and some bugs crossing the 2.5 cm wide insecticide dust.

Experiment II: Effect of Relative Humidity on the Efficacy of Insecticide Dusts

Similar to Experiment I, 15 by 15 cm vinyl tiles were used as the substrate material. Each side of the tile had a 5.08 cm wide band. Cimexa dust was applied to each of the 4 bands through a mesh (size of opening: 0.067 mm^2) to allow for even distribution of the dust (Figure 2). A total of 0.126 g of Cimexa was applied (total area: 206.45 cm^2) based on the label rate (0.61 mg/cm^2). A wider dust band was used in this experiment to

avoid too low mortality of the bed bugs after aging the treated tiles following exposure to moisture.

Three different relative humidities were created using saturated salt solutions as described by Greenspan (1977). They were 50%- Mg (NO_3).6$H_2$0, 75%- NaCl, and 100%- K_2SO_4. They represented three different relative humidity levels ranging from normal to extreme environmental conditions. Relative humidity in each container was confirmed by placing a HOBO External Temp/RH data logger (Onset Computer Corporation; Bourne MA) in each treatment. The actual RH recorded by HOBO dataloggers in the three containers were 52%, 75%, and 100% respectively. Each container (47 cm × 37.8 cm × 28.3 cm, Sterilite Corporation, Townsend, MA) with ten tiles and saturated salt solutions (400 ml) were kept in an incubator at 26^{o}C. After one month and two months, tiles were removed from the humidity condition and used for treatment immediately. The control (dry dust) was applied to the tiles on the same day of treatment. In the negative control, no dust was placed on the tiles. Each treatment combination had 5 replications.

Two days prior to the treatment, 22 bed bugs (11 males and 11 3rd-5th instars nymphs) were placed in each petri dish as described in Experiment I. Bed bugs were fed 4 and 6 d prior to the experiment for one and two months aging period respectively. Each tile was placed on a piece of cardboard paper inside a plastic container with a plastic ring of (3.8 cm diameter and 1.3 cm height) at the center of the tile (similar to Figure 3.1). The bed bugs were exposed to the dust treated panels in the same manner as in Experiment I. The first 20 bed bug that crossed the treated band were removed from the arena using featherweight forceps and placed into a petri dish and stored in an incubator. For each

replicate, the time taken to cross the treated Cimexa band was recorded for 20 bed bugs. It took 2.15 to 6.5 minutes to cross the dust band (from the first bed bug touching the inner border of the band to the 20th bed bug crossing the outer border of the band). Bed bug mortality was recorded daily at 1-7, and on 10, 12, 14 and 21 days after exposure. Dead bed bugs were removed from the dishes after each examination.

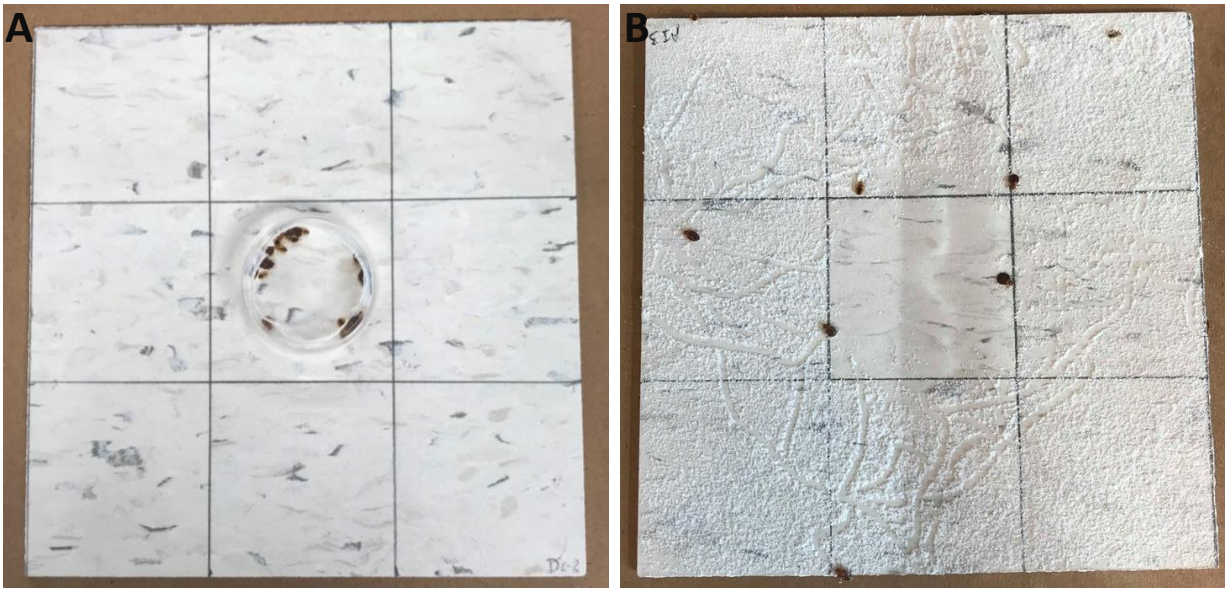

Figure 3.3. Experimental setup in the relative humidity experiment. A. bed bugs were confined in the center of a 15 by 15 cm vinyl panel with a plastic ring, B. bed bugs on a panel with a plastic ring removed and some bugs crossing the 5 cm wide insecticide dust band.

Statistical Analysis

Corrected mortality was calculated by using Abbott's formula (Abbott, 1925). Analysis of variance (ANOVA) was used to analyze the percent corrected mortality data from each treatment. The data were checked for normal distribution and transformed if they were not normally distributed. Kruskal-Wallis test was used for data that are not normally

distributed even after transformation. Tukey's HSD test was used to separate means of percent mortality. All statistical analyses were performed using SAS software (version 9.3) (SAS, 2011).

Results

Experiment I: Effect of Steam on Efficacy of Insecticide Dusts

In the negative control, the mortality at 10 d was 6%. At 7 d, dry Cimexa and Cimexa exposed to steam (hereafter referred to wet Cimexa) caused 93 ± 3% and 59 ± 7% (mean ± SE) corrected mortality, respectively (Figure 3.3 (A)). They were significantly different ($F = 19.11$; $df = 1$; $P = 0.002$). At 10 d, dry Cimexa and wet Cimexa caused 100 ± 0% and 76 ± 7% corrected mortality, respectively. They were significantly different ($F = 12.87$; $df = 1$; $P = 0.007$). These results indicate that moisture from steam exposure significantly reduced the efficacy of Cimexa dust.

Steam also significantly reduced the efficacy of Alpine dust (Figure 3.3 (B)). At 7 d, dry Alpine caused 55 ± 4% corrected mean bed bug mortality whereas Alpine dust exposed to steam (hereafter referred to wet Alpine) caused 34 ± 3% corrected mean mortality ($F = 19.09$; $df = 1$; $P = 0.002$). At 10 d, dry Alpine caused 69 ± 3% corrected mean bed bug mortality which was significantly higher than that (43 ± 4%) caused by wet Alpine ($F = 23.11$; $df = 1$; $P = 0.001$).

Similarly, dry Tempo dust caused higher bed bug mortality than Tempo dust exposed to steam (hereafter referred to wet Tempo). At 7 d, dry Tempo caused 44 ± 4% corrected mean bed bug mortality whereas wet Tempo caused 25 ± 4% corrected mean bed bug mortality ($F = 10.61$; $df = 1$; $P = 0.012$). At 10 d, dry Tempo application caused

48 ± 4% corrected mean bed bug mortality which was significantly higher than that (30 ± 5%) by wet Tempo (F = 6.53; df = 1; P = 0.004).

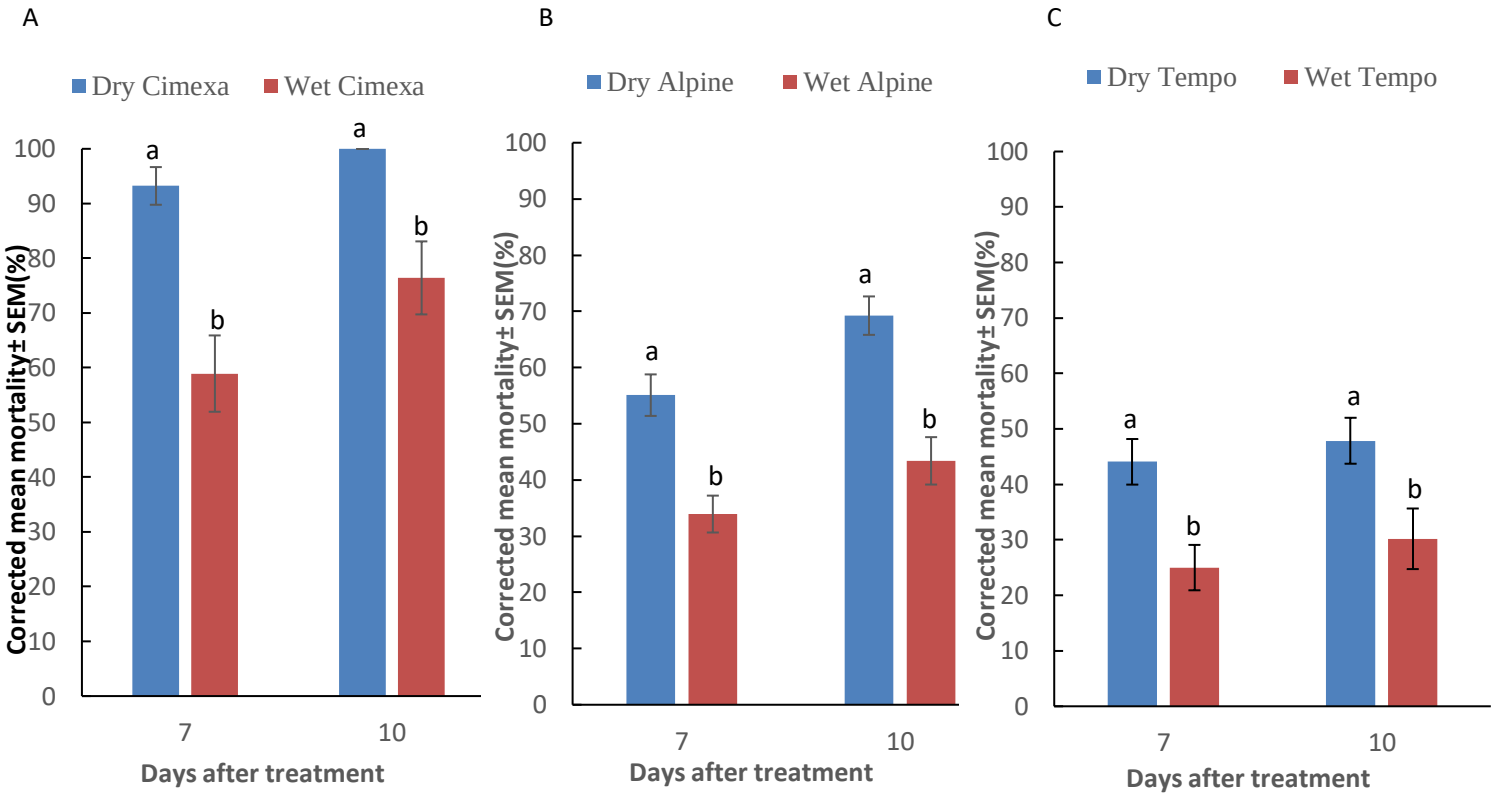

Figure 3.4. Effect of steam on the efficacy of (A) Cimexa, (B) Alpine, and (C) Tempo against bed bugs. "Dry Cimexa" were not exposed to steam. Means with different letters are statistically different (P < 0.05, Tukey's HSD test).

Among the three insecticides dusts, the efficacy of dry dust was greatest for Cimexa followed by Alpine (Figure 3.4) and Tempo (F =74.19; df = 2; P = 0.0001); the same was true for the efficacy of dust after exposure to steam Cimexa > Alpine > Tempo (F = 18.7; df =2; P = 0.0002).

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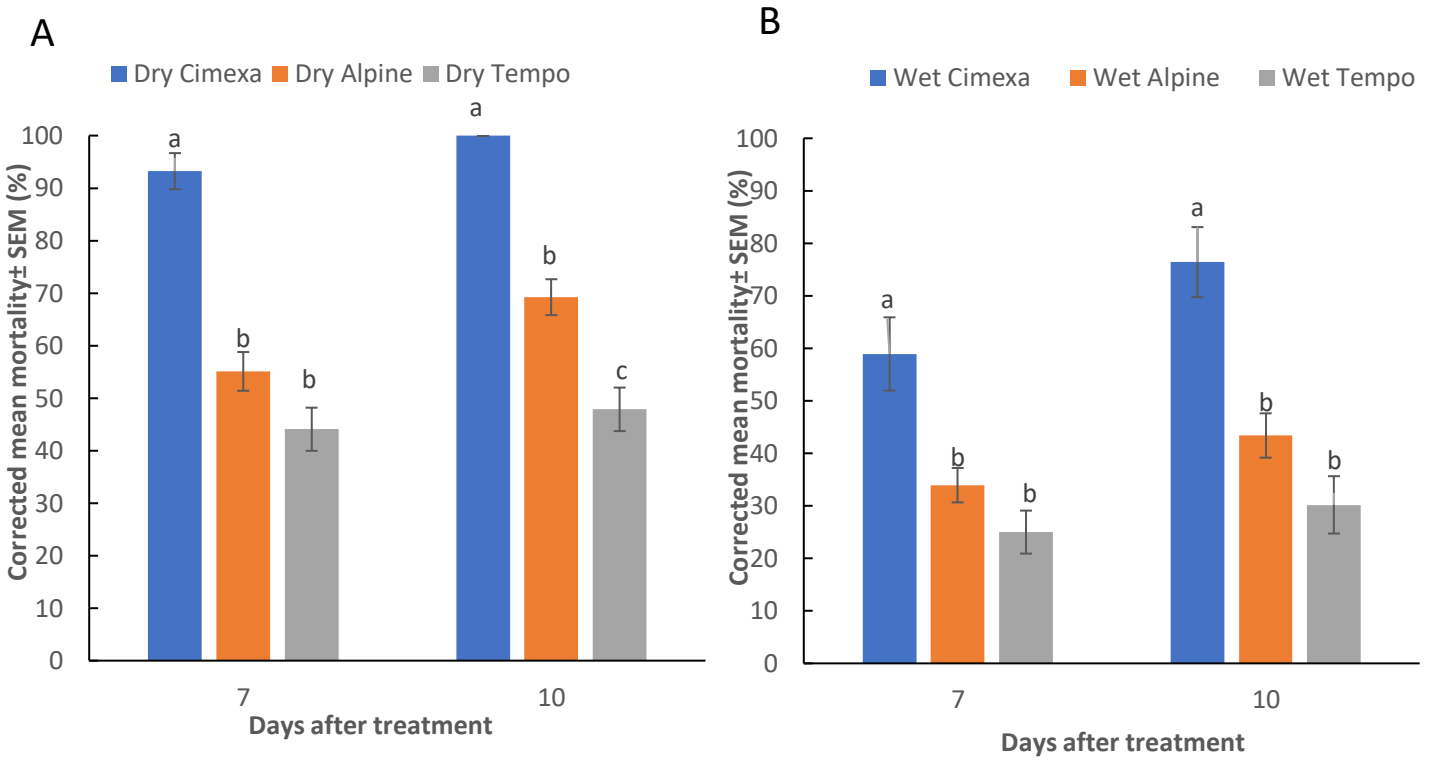

Figure 3.5. Efficacy of selected insecticides against bed bugs (A) Dry Condition (B) Wet Condition. Means with different letters are statistically different ($P < 0.05$, Tukey's HSD test.)

Experiment II: Effect of Relative Humidity on the Efficacy of Insecticide Dusts

In the first test where tiles were aged for one month under different RH conditions, the control mortality was ≤ 5% at different relative humidity conditions at 10 d after treatment. At 7 d, fresh Cimexa caused 98 ± 3% corrected mean mortality. Cimexa dust aged for one month under 52, 75, and 100% RH caused 65 ± 8%, 58 ± 6 %, and 38 ± 10% corrected mean mortality, respectively. There were significant differences among the treatments ($F = 11.23$; $df = 3$; $P = 0.0003$) (Figure 3.5A). No significant difference was found among the corrected mean mortality caused by the Cimexa aged under one month under 52, 75, and 100% RH at 7 d. At 10 d, fresh Cimexa caused 99 ± 1% corrected mean mortality whereas Cimexa aged for one month under 52, 75, and 100%

RH caused 81 ± 6%, 79 ± 6%, and 56 ± 8% corrected mean mortality respectively ($F = 17.1$; $df = 3$; $P = 0.001$). The 100% RH treatment caused significantly lower mortality than the other treatments.

In the second test where tiles were aged for two months at various moisture, the control mortality was ≤ 6% at different relative humidity conditions at 10 d after treatment. At 7 d, fresh Cimexa caused 68 ± 6% corrected mean mortality, Cimexa aged for two months under 52, 75, and 100% RH caused 55 ± 6%, 45 ± 8%, and 28 ± 6% mean mortality, respectively (Figure 3.5B). They were significantly different ($F = 6.82$; $df = 3$; $P = 0.0036$). Cimexa aged for two months under 100% RH caused significantly lower mortality than other treatments. At 10 d, fresh Cimexa caused 83 ± 4% corrected mean mortality; Cimexa aged for two months under 52, 75, and 100% RH caused 60 ± 6%, 60 ± 3%, and 39 ± 6% mortality, respectively. Mortality from these treatments were fresh Cimexa > 52% and 75% RH > 100% RH ($F = 13.18$; $df = 3$; P .0001)

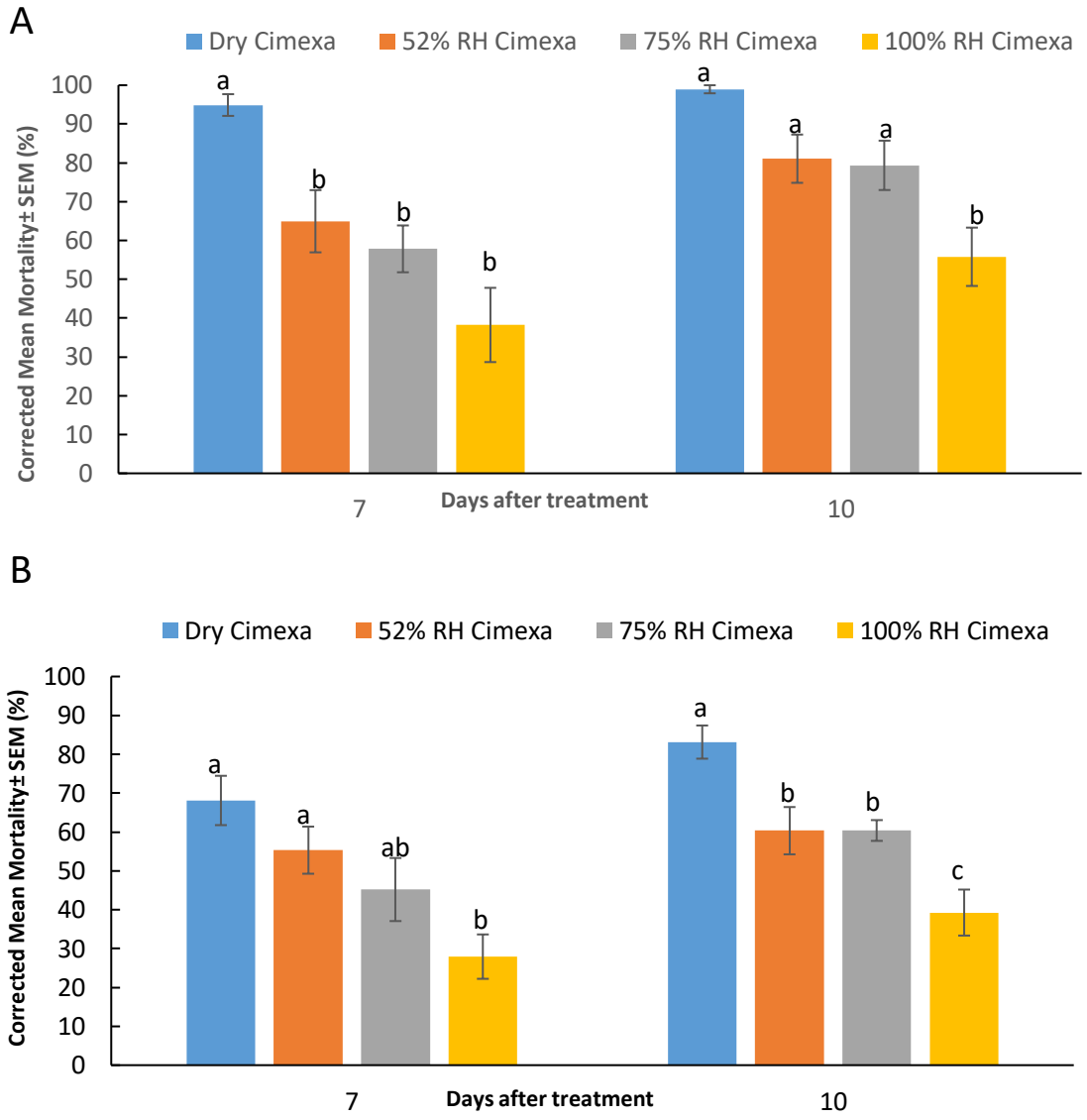

Figure 3.6. Effect of relative humidity on the efficacy of Cimexa aged for (A) one month and (B) two months. Means with different letters are statistically different ($P < 0.05$, Tukey's HSD test).

Discussion

This study clearly shows that environmental moisture affects the performance of insecticide dusts used against bed bugs. Insecticide dusts aged at 52 to 100% RH for one month exhibited lower efficacy. It is common that residential homes have around 50% RH level for at least some periods of the year. These data suggest field performance of

the insecticide dusts may be significantly lower compared to that obtained under laboratory conditions. Also, in the field dust is often applied in cracks and crevices that may have slightly different RH compared to ambient RH. Further studies can be done by applying dust in micro-environment. The relative efficacy of the three commonly used insecticide dusts evaluated in the study against *C. lectularius* was Cimexa > Alpine > Tempo.

Mode of action of each insecticide dust is very important in understanding the reason that some insecticide dusts are more effective than others. Inorganic insecticides such as diatomaceous earth (DE) dust and silica gel cause desiccation and death by removing the ultra-thin, protective layer of wax from the cuticle of an insect (Potter et al., 2014). Neonicotinoids, such as dinotefuran in the Alpine dust, mimic the agonist action of acetylcholine at nicotinic acetylcholine receptors (Le Patourel, 1986) which leads to overstimulation of the nervous system causing involuntary muscle contraction, cessation of feeding, paralysis, and ultimate to death (Bloomquist, 2009; Boase, 2001). Pyrethroids and pyrethrin are sodium channel modulators that cause paralysis of the insect (IRAC, 2016). Most of the examined field bed bug populations are resistant to pyrethroids (Davies et al., 2012). Silicon dioxide based (SiO_2) desiccant dusts are popular because of its low mammalian toxicity and long residual life (in dry environments). Dust formulations containing silica gel or DE are effective against pyrethroid-resistant bed bugs (Romero et al., 2007). DE is both sorptive and abrasive (Ebeling, 1971) but it has been found to be less effective than non-abrasive silica gels for various pests, even when applied at higher rates (Ebeling, 1971). This may be the reason that Cimexa was the most effective dust against bed bugs (Singh et al. 2016).

There are some studies about the effect of moisture on the efficacy of insecticides. Diatomaceous Earth (DE) loses its effectiveness when used under highly humid conditions or when water is offered ad libitum to German cockroaches (Faulde et al., 2006). As mentioned earlier, the mode of action of DE is generally recognized as a desiccant effect on insects. However, some studies have shown that some dusts are not affected by relative humidity. The toxicity of boric acid, desiccant, or conventional insecticide dusts against German cockroaches was not affected by relative humidity (Ebelinc et al., 1967). The tolerance of red flour beetle species to the sorptive silica dust treatments was found to be increased with increasing moisture content (Le Patourel, 1986). For German cockroaches, boric acid and other insecticidal dust formulations can be used effectively in a wide range of moisture conditions. Moisture in the form of relative humidity does not strongly affect the toxicity of dust formulations against German cockroaches (Appel et al., 2004). Ingested Boric acid in German cockroaches caused death maybe ultimately by starvation via alteration of midguts (Habes et al., 2006). Based on these studies, we can see conflicting results about the effect of moisture in the efficacy of insecticide dust. The variation in these reported studies might be attributed to the fact that different dust formulations/insecticides tested have different modes of action. Based upon their mode of action, the effect of moisture in the efficacy of insecticide dust may be significant or non-significant. My study shows the efficacy of desiccant (silica gel) was negatively affected by increased moisture content. This is plausible as moisture negatively affect the ability of dust particles to be attached to insect cuticle. And neurotoxin insecticide like pyrethroid and neonicotinoids, can be unstable in moist condition.

Applying steam is a commonly used effective method for treating bed bug infestations (Wang et al., 2009). From my study, exposure to steam will affect the performance of insecticide dusts. So, in the field, dust should not be applied until areas treated with steam have been completely dried. Also, it's better to avoid mopping floors in areas where dust has been applied shortly after dust treatments.

In this study, mortality caused by Cimexa dust band was slower or lower than that reported by Singh et al. (2016). It may be due to the batches of Cimexa used in experiments or different strains of bed bugs. We found significant differences in efficacy among the batches of Cimexa (unpublished data). In my experiment, there was a significant difference among the mortality caused by the fresh Cimexa used for one and two months though they were taken from the same bottle of product. Though we used the same strain in both experiments, the age of the bed bug males used in the experiment was unknown. Handling procedures may have contributed to the variance. The product properties may have changed over time. Further studies can be done to evaluate changes in product effectiveness over time. Further, different exposure methods and more replicates can be explored to increase the consistency of experiments.

In summary, this study revealed the impact of moisture from steam or differences in the natural environment on insecticide dust effectiveness against bed bugs. It provides guidance on how to effectively use insecticide dust treatment in bed bug management. Effective use of insecticides can be crucial to combat bed bugs from encroaching our homes. Further studies on the effect of moisture on the physical and chemical properties of insecticide dusts is recommended. Development of an optimum treatment plan in a wide range of moisture conditions will also be important.

Acknowledgments

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CONCLUSION

My first study showed that most of the bed bug strains evaluated in the study had moderate to high resistance of bed bugs against different insecticides: Suspend SC (deltamethrin), Transport GHP (acetamiprid + bifenthrin), Temprid SC (imidacloprid + β-cyfluthrin), and Tandem (λ-cyhalothrin + thiamethoxam. Dry residue of Phantom SC was deemed ineffective against all bed bug strains in my study. The results show insecticide sprays have limited effectiveness for the control of bed bugs when used alone, so using only insecticide sprays alone to control bed bug is not suggested. Adoption of integrated bed bug management strategies that combine both chemical and non-chemical methods is recommended to reduce insecticide selection pressures and delay resistance development. New tools and techniques should be developed for the sustained management and control of the bed bug problem. My second study concluded that the efficacy of various insecticide dusts used against bed bugs are affected by the moisture. Due to this, moisture should be considered as one of the important factors during the insecticide dust application. Exposure to steam significantly reduced the efficacy of all three insecticide dusts: Cimexa (92.1% amorphous silica gel), Alpine (0.25% dinotefuran, 95% diatomaceous earth) and Tempo (1% cyfluthrin) suggesting that in the field, dust should not be applied until areas treated with steam have been completely dried to minimize the effect of moisture from steam treatment. Cimexa caused higher mortality as compared to Alpine and Tempo dust. Further, Cimexa treated tiles that were aged under different (52%, 75%, and 100%) RH for one and two months caused significantly lower mortality to *C. lectularius* than dry Cimexa. It is important to users when choosing products in a high moisture environment. Our results could help to determine the

parameters where dust could work best. Optimum treatment plan should be developed in a wide range of moisture conditions.